# 1

# Courtship Behavior of the Queen Butterfly, *Danaus gilippus berenice* (Cramer) [1,2]

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(Plates I-VII; Text-figures 1-11)

## CONTENTS

## I. INTRODUCTION

THE study of sexual behavior of the Lepidoptera has contributed significantly to the development of biology, particularly in the fields of ethology (Baerends, 1959; Tinbergen, 1951), sexual selection (Richards, 1927; Fisher & Ford, 1928; Brower, 1963), and chemical communication (Gotz, 1951; Karlson & Butenandt, 1959; Wilson & Bossert, 1963). Nevertheless, entire courtship patterns have been described for only a few species, and our knowledge of the roles that visual, tactile, chemical and acoustic stimuli play is severely limited. This is more true of the moths than the butterflies, but even in the latter few adequate studies exist, and it is with butterfly courtship that this paper will be concerned. When one considers the diversity in flight behavior, scent disseminating systems, color, pattern, size and sound-producing organs in these insects, the prospects for comparative analyses are indeed fascinating.

There are three principal reasons why so few comprehensive studies have been made: first, entire courtships ending in copulation are rarely seen in nature; second, when mating does take place, the rapid series of complex events makes recording extremely difficult; and third, there

1

has been a remarkable lack of systematic experimentation in the field, laboratory or insectary. On the other hand, there are numerous fragmentary references to pairs which have already mated (Carpenter, 1935) and to incomplete courtships, but in fact interpretations of the latter have often been incorrect, as Lederer (1960) has pointed out.

Some of the best analyses of butterfly courtship have been done in the confinement of outdoor cages but the work of Crane & Fleming (1953) and Crane (1955, 1957) has shown that great care must be taken to be sure that the insects have enough space, proper lighting, protection from wind and sufficient foliage to allow their behavior to be normal. One example of the possible pitfalls of this method was described by Tinbergen, Meeuse, Boerema & Varosieau (1942) in their experiments on the role of the scent patches on the forewings of the male Grayling butterfly (*Eumenis semele* L.). An initial finding that the removal of these had no effect proved on closer study to be an artifact of crowding. Similarly, Stride (1958a) found that unreceptive *Hypolimnas misippus* Linnaeus females flew into the cage roof when pursued by their males and were unable to exhibit their normal evasive behavior. Moreover, in our work with caged Queen butterflies, we found that the behavior of both sexes is incomplete and that the males often trapped the females in corners where copulation occurred.

Another valuable approach has been to present males with dead butterflies, artificial dummies or living butterflies that were restricted in various ways. These methods have proved particularly useful in elucidating the stimuli involved in the approach reaction of the male to the female, and include attaching the dummies and living or dead male or female butterflies to wand-like rods (Tinbergen *et al.,* 1942; Crane, 1955; Stride, 1956, 1957, 1958a, b), pinning them to flowers (Magnus, 1950), tethering them at the end of threads (Tinbergen *et al.,* 1942; Brower, 1958) and tying them to leaves (Lederer, 1960).

This paper will present the results of another method of investigating courtship in which laboratory-reared female Queen butterflies, *Danaus gilippus berenice* (Cramer), were released to wild male Queens in their natural environment. It is hoped that the new techniques developed for obtaining the data in a form that can be treated statistically will prove a stimulus to further experimental research. The courtship behavior of the Queen butterfly will also be compared as far as is possible with that of other butterflies of the subfamily Danainae as described in the literature.

No attempt will be made to compare the courtship of these butterflies with species of other taxonomic divisions of the Rhopalocera since this is to be the subject of a later publication. Of particular interest is the major role that scent appears to play in the courtship of the Danainae. Consequently, the paper will summarize the literature on the morphology and histology of the scent organs and will critically evaluate the inferences as to their function that may be drawn from their structure. As will be seen, the behavioral system in the Danainae that depends upon scent stimuli opens a new area of investigation in which it may be possible to reconstruct the evolution of a chemical language that prevents interspecific hybridization in nature.

## II. Acknowledgments

## III. GEOGRAPHIC DISTRIBUTION AND TAXONOMY OF THE DANAINAE

The Queen butterfly is a member of the family Nymphalidae and belongs to the mainly tropical subfamily Danainae. This subfamily is divided into three tribes: the Lycoreini, whose members occur only in the Neotropics and West Indies; the Euploeini, whose members are limited almost exclusively to the Indo-Australian region; and the Danaini, whose members are pantropical with a few species extending into temperate regions (Seitz, 1908; Fruhstorfer, 1910; Aurivillius, 1911). In this paper the terms lycoreine, euploeine and danaine will be used in specific reference to these tribal divisions.

The taxonomic relationships of the Old and New World danaines have never been satisfactorily worked out, although Forbes (1939) has made the most significant contribution in this respect. The reasons for this arise from nomenclatorial difficulties, the fact that taxonomists have worked mainly with either New or Old World material, and because the tribe is almost certainly in a rapidly expanding phase of its evolution.

It should be noted that the three best known species of danaines which are often referred to the genus *Danaus* are generically distinct. The Monarch butterfly, familiarly known as *"Danaus plexippus,"* is in fact a member of a monotypic, New World genus, and should be called *Anosia erippus* (Cramer). It appears to occur as three subspecies: *Anosia erippus erippus* (Cramer) in southern South America; *A. e. menippe* (Hübner) in North America; and *A. e. megalippe* (Hübner) in the intervening areas. The second species is *"Danaus chrysippus"* which has been placed in another monotypic genus that occurs widely in the Old World and is called *Limnas chrysippus* (Linnaeus). Finally, the Queen butterfly, *Danaus gilippus* Cramer, is a New World member of the polytypic genus *Danaus* which has a pantropical distribution. The Old World *Danaus,* the New World *Danaus,* and *Limnas* appear more closely related to each other than any of them is to *Anosia,* the Monarch.

According to Forbes, *Danaus gilippus* is one of four New World *Danaus* species. Two of these, *D. gilippus* and *D. eresimus* Cramer, are widely distributed and occur as a number of subspecies in the West Indies, Central and South America and parts of southern North America. The third, *D. cleophile* Godart, is limited to the island of Hispaniola while the fourth, *D. plexaure* Godart, is found only in South America.

The subspecies studied in the present investigation was mainly *Danaus gilippus berenice* (Cramer). This occurs throughout Florida, west to Texas, and also in Cuba, but the latter population may prove to be a distinct subspecies. It has yet to be determined whether some of the subspecies are in fact true species. For example, *D. g. berenice* from Florida is very distinct in color from the Trinidad *D. g. xanthippus* Felder, and both differ in color and size from the Jamaican *D. g. jamaicensis* Bates. Some of the photographs are of the Trinidad subspecies, and reference will be made to this form on several occasions.

## IV. SCENT ORGANS OF THE DANAINAE

The Danainae have attracted the attention of numerous investigators ever since Fritz Müller (1877b) speculated upon the functional relationship of two elaborate organs found in male *Danaus* species. These are paired hairpencils which the male can extrude from the end of his abdomen, and pockets located on the upper surface of the hindwings (Plate I). In discussing the pockets, he said, "the position and shape of these sexual cavities is such that the extremity of the abdomen might easily be applied to them, and as the hairs of the abdominal organs unite in the form of a brush, it would not be impossible, or even difficult, to introduce them into the depths of the cavity." (p. 619).

Thus began the biological mystery which enshrouds the physiological relationship of the hairpencils and wing glands. To provide a basis for solving this, the variation in occurrence, position and structure of these two organs in the systematic divisions of the subfamily Danainae will be reviewed. This will be followed by a brief survey of the species so far studied, and then by a detailed comparative examination of the morphology and histology of both organs. Following this there will be a discussion of the sexual scents, and finally a summary of the evidence that the males perform a solitary behavior in which they actually apply their hairpencils to their wing glands.

### (A). *Variation Within the Subfamily*

As far as is known, the males throughout the entire subfamily Danainae possess a pair of abdominal hairpencils with the exception of the species in the danaine genus *Ideopsis* which have none, and those of the euploeine genus *Hestia* which have four (Müller, 1877a; Seitz, 1908; Haensch, 1909; Fruhstorfer, 1910; Forbes, 1939; Wheeler, 1946; Talbot, 1947). In contrast to the hairpencils, the glands on the wings of the males are not found in all three tribes, and there is considerable variation both in their

form and position. Thus in the Lycoreini they are altogether lacking. In the Euploeini they occur on the hindwings of some species, the forewings of others, and are absent from still others. In the Danaini they are present on the hindwings of all species and are usually concentrated in the form of a single patch *(Amauris* and *Parantica),* a more complex pocket *(Danaus* and *Limnas),* or an even more complex pouch *(Tirumala).* Sometimes, however, they occur diffusely *(Radena)* or in the form of an undulating band *(Ideopsis).* Because of these morphological differences, wing gland will be used as a general term, and wing patch, wing pocket and wing pouch will be used as specific terms. As will be seen, this terminology reflects their different modes of origin in development from the pupal to adult stage.

### (B). *Species Previously Studied*

No histological investigations have yet been made upon the lycoreines. Illig (1902) described and figured the hairpencils of an undesignated species of *Euploea,* Freiling (1909) the hairpencils of *Euploea asela* Moore, and Eltringham (1915, 1935) the hairpencils of four euploeines: *Euploea core asela* Moore, *Trepsichrois mulciber* Hübner, *Tronga brookei* Moore and *Hestia lynceus* Drury. Eltringham also described the patches on the forewing of the first of these and on the hindwing of the second; the other two lack areas of specialized scales on their wings. For the danaines, Eltringham (1913, 1915) has investigated the wing glands and hairpencils of eleven species: *Amauris niavius* Linnaeus, *A. psyttalea* Plotz, *A. egialea* Cramer, *A. ochlea* Boisd., *A. hecate* Butler, *A. whytei* Butler; *Tirumala petiverana* Doubleday, *T. limniace* Cramer; *Parantica eryx* Fab.; *Limnas chrysippus* (Linnaeus), and *Danaus lotis* Cramer. Müller's (1877b) original paper gave a preliminary histology of the wing pockets of the southern South American Monarch butterfly. Those of the North American Monarch have been superficially investigated by Hausman (1951) and Urquhart (1958, 1960). It is presumably upon this subspecies that Illig (1902) carried out his detailed study of both organs. Illig also studied *L. chrysippus,* as did Eltringham (1915). Freiling (1909) described and figured the hairpencils of the African *Danaus septentrionalis* Butler in great detail, but did not investigate the wing pockets of this species. The only remaining danaine which has been studied is the South American subspecies of the Queen butterfly (*D. gilippus gilippus* Cramer). Müller (1877b) compared the hairpencils and gross histology of the wing pockets with those of the South American Monarch.

### (C). *Structure and Histology of the Abdominal Hairpencils*

With the exceptions noted above, the hairpencils of the Danainae are paired organs lying laterally inside the abdomen at the end of the body and have arisen through invagination of the intersegmental membrane between the 8th and 9th sternites (Ehrlich, 1958). They have been given a variety of names which allude to their structure or function. "Hairpencil" refers to the cylindrical bundle of individual hairs; "abdominal" or "extrusible brush" to the fact that when partially extruded it looks like a small artist's paint brush; "brush bag" to the structure when normally retracted in the body, and "duftpinsel" because the brush is scented. They have also been called "anal scent glands," which is a misleading term because they are neither connected to nor derived from the digestive system.

The male butterfly extrudes both hairpencils simultaneously by an increase in the pressure of its abdominal body fluids. While various muscles come into play to bring this about, as far as is known there is no direct muscular control of the extrusion. On the other hand, there is a large retractor muscle attached to the base of each hairpencil which is mainly responsible for retracting the organ (Plate VI, figure 1). They can also be forced out by carefully squeezing the posterior part of the male's abdomen. Plate I, figure 1, shows the abdomen of a male Queen butterfly prior to being squeezed and the hairpencils in their normal, completely withdrawn position. Plate I, figures 2 and 4, show them extruded to an extent of about 75%. Because the individual hairs are attached only at their bases, the further out the pencil is forced, the more it tends to splay. Plate I, figure 3, shows the hairpencils completely everted. During courtship, the Queen males sometimes extruded them as far as this (Plate II, figure 1), but not always (Plate II, figures 2-3). In *Lycorea ceres ceres* (Cramer) which we have observed in Trinidad, the hairs, unlike those of the Queen, are extruded spontaneously when the living males are handled (Plate V). This apparently also occurs in the lycoreine genus *Ituna* (Müller, 1878) and in the euploeines (Fruhstorfer, 1910), which suggests that the hairpencils in both these tribes play an additional role in protection, while in danaines they are used solely in courtship.

The hairpencils of the Queen are about 4 mm. long and .75 mm. in diameter (Plate I, figures 2 and 4). Those of the Monarch are much smaller, while those of euploeines and lycoreines are considerably larger, being up to 12 mm. in length in one species of *Lycorea* (Illig, 1902).

Except for the modifications discussed below, each hairpencil consists of a bundle of hundreds of scales which have become elongated as more or less hollow hairs. Each of these hairs is continuous with one very large trichogen secretory cell embedded in the basal tissue of the organ (Plate VI, figure 1). Our own observations and those of Illig, Freiling and Eltringham lead us to accept the hypothesis that a secretion produced by these basal cells flows up their respective hairs and diffuses out or oozes through small pores along their length. Presumably, the minute globules between the hairs (Plate VI, figures 1 and 2) represent this secretion. These globules were observed by Illig in *"Danaus plexippus,"* by Freiling in *D. septentrionalis* and *Euploea asela* and by Eltringham in *L. chrysippus* and *D. lotis,* although not in *E. core asela.* This conflict with Freiling in regard to the latter butterfly probably reflects a difference in their histological techniques, but it could also be that they were dealing with different species. Illig's findings that the globules are released at the base of the hair where it enters the cell is almost certainly an artifact of his preparation. Eltringham (1915) provided evidence that the globules in *L. chrysippus* are not chitinous since they readily dissolve in eau-de-javelle.

Eltringham (1915, 1929) was unwilling to accept the view that the hairpencils are secretory organs in *Limnas, Danaus, Amauris* and *Tirumala,* partly because he could find no glandular cells in the hairpencils of *Limnas* and due to his conviction that these organs become charged with a secretion from the wing glands. However, we have found that the globules are present between the hairs of sections of Queen hairpencils preserved prior to the time the male emerged from its pupa. This new evidence, together with that of Brower & Jones (1965, see section IV-E-3), proves that the hairpencils are independent secretory organs in the Queen butterfly, and it seems probable that they are in all danaines.

We have also examined microscopically the hairpencils of living Trinidad Queen males from the time of hatching until several days old and in all a black dust-like material was seen densely packed between the hairs. Presumably these particles are the analogs of the globules just described. One wonders if they evaporate *in situ* during the hairpencilling behavior or if they shower forth as a rain of scented dust.

Brief mention will now be made of the hairpencils found in the remaining danaine genera as described by Eltringham (1913, 1915). The species of the genus *Tirumala* have only a single type of hair, which is basically similar to those in *Limnas* and *Danaus.* In *Parantica* as well, only one kind of hair is found, but these exhibit a further specialization in that distally they bear small leaf-like structures which readily break off to produce small particles. The function of these is apparently similar to the particles produced in much larger amounts in *Amauris.* The species of the latter genus are far more complex than all the other danaines. For example, in *Amauris niavius* there are three different kinds of hairs: those arising from the base of the gland, which are light in color and form one tuft; those arising more distally on one side of the gland, and forming an adjacent black tuft; and those arising from the central part of the organ. The central hairs are long, delicate, threadlike structures segmented along their length. These break up to produce numerous minute particles which are found densely packed between the other hairs and are presumably wafted into the air when the hairpencils are extruded. Eltringham thought that the hairs of the black tuft are stiffer than those of the light-colored ones and probably function to assist in lifting the scales covering the wing patch, thereby allowing the entire (?) brush to be charged with secretion. In other species, the stiff hairs are surrounded by the light ones and form a central core. In *Amauris egialea* the fragmenting hairs appear to be absent, but a fourth quite different type is found arising from the base of the organ and together they form a cone protruding up into the center of the light-colored hairs. These may not be hairs at all, but rather scent-producing cells. However, in *Amauris ochlea* a slightly different cone is present and apparently does produce fragments, and similar structures are present in other species of *Amauris.*

Other variations on these basic themes are seen in the hairpencils of the Euploeini, which, however, do not produce the dust particles. The genus *Hestia* is particularly remarkable in that stiff hairs are apparently completely separated from softer ones, with the result that the insect has four hairpencils instead of two. It appears from the observations of Illig (1902), Freiling (1909) and Latter & Eltringham (1935) that most of the euploeines possess two types of hairs so arranged that the more distal ones splay out at right angles to the proximal, inner ones, giving an appearance similar to that of a daffodil flower (see Freiling, Plate 16, figure 36).

Although Eltringham (1915, 1935, *in* Latter & Eltringham) doubted that the hairpencils were secretory organs in some danaine species, he

held the opposite opinion for the euploeines. In describing *Tronga brookei,* he said, "this species has no brands or patches of scales on the wing, and the brushes must therefore perform their scent-producing functions unaided" (1915, p. 172). It is evident from this statement that his reasoning was more strongly influenced by gross morphology than glandular details. This in turn undoubtedly resulted from the limited amount of preserved material at his disposal and it is a tribute to him that he was able to produce as much information as he did.

In the lycoreines there are no wing glands and since the hairpencils are odoriferous (section IV-E) these organs must both produce and disseminate the scent.

To summarize, the hairpencils seem definitely to be secretory as well as scent-disseminating organs in at least some species of all three tribes of the subfamily. In the danaine genera *Amauris* and *Parantica,* certain hairs are further specialized to produce fragments which mechanically aid the scent-disseminating function of the hairpencil. As Eltringham emphasized 30 years ago, there is still considerable uncertainty associated with these organs, and it is clear that further investigation of both their microstructure and secretory activity is greatly needed. Besides the specific variation, factors to be considered are changes that occur in the development and aging of individuals as well as observations of the organs in living as opposed to preserved material. Electron microscope examination of the hairs and their gland cells along the lines carried out by Barth (1960) on pierids would also be of extreme interest in elucidating their structure and function.

### (D). *Structure and Histology of the Wing Glands*

The following paragraph, taken nearly *verbatim* from Müller (1877b, pp. 616-617) gives an excellent picture of the external morphology of the wing pockets of the South American Queen and Monarch butterflies *(Danaus g. gilippus* and *A. e. erippus)*: they are visible on both sides of each hindwing as a small swelling but are more prominent on the upper surface (Plate I, figures 1 and 2). They are elliptical in shape and lie closely parallel and posterior to the second cubitus vein. In the Queen, each is about 4 mm. long, by 1.5-2 mm. wide, while in the Monarch they rarely exceed 2 mm. in length by .6 mm. in width, even though the Monarch is the larger of the two species. The opening of the pocket is on the upper surface of the wing. An area "denuded" of scales is visible near the opening (Plate I, figure 2)

which Müller speculated resulted from abrasion by the hairpencil as it was pushed into the pocket.

The developmental origin of the wing pocket sheds considerable light on its structure. Approximately half of it arises through an evagination of part of the upper wing membrane. When a male has just emerged from its pupa, this evaginated areas exists as a small flap-like projection (Plate VII, figures 1 and 3), but as the wings expand, this folds over the unevaginated portion and forms the pocket (Plate VII, figures 2 and 4); to avoid confusion, it should be noted that figure 1 is a section through a right wing pocket and figure 2 a left wing pocket.

The inside of the pocket is lined with small flat scales (Plate VII, figure 2). From our studies of the Queen and those of previous authors on other species, it is evident that these scales arise from large cells which are arranged in alternating rows with smaller cells, each of which may (as in the Queen) or may not have a very small filiform scale projecting from it. The functional difference of these two cells and their associated scales is not at all clear, but it is certain that we are here dealing with an active secretory organ. Evidence of this is seen by comparing the section made through the pocket 10 minutes after the male had hatched (Plate VII, figure 1) with that of the 24-hour-old male (Plate VII, figure 2). This shows that the cells of the flat scales greatly enlarge during the first day in the adult male's life to fill nearly the entire space between the upper and lower membranes. Presumably secretion issues forth from one or the other or both of the cell types through the stalks of their respective scales and accumulates so that it can interact with the hairpencils when they are inserted. Note also that the flat scales are arranged in such a way that they will offer the least resistance to the hairpencil during its insertion.

Certain of the conclusions that Urquhart has made in his study of the Monarch butterfly are incorrect. Without reference to Illig, Freiling or Eltringham, he stated that a fluid, originating in the wing vein adjacent to the pocket, flows between the upper and lower wing membranes and fills what he terms the "cavity" of the wing pocket, "where it becomes transformed into a white, spongy, wax-like substance" (1958, p. 10; 1960, pp. 53 and 151). By "cavity," Urquhart is referring to the collective glandular tissue of the wing pocket, and not to its large, obvious air space. He thus erroneously interprets the gland cells as being an amorphous mass. Moreover, it is highly doubtful that this vein

supplies anything to the pocket that it does not give to other non-specialized areas of the wing (Illig, 1902). This seems verified for the Queen butterfly by Plate VII, figure 2.

Although the wing glands of the danaine genera *Amauris and Parantica* are less complex patches, their histological structure is very similar to the pockets of *Danaus* and *Limnas*.

This, however, is not the case with the danaine genus *Tirumala*. In these butterflies, the wing gland is a pouch which has arisen by invagination of the upper wing membrane and forms a large space between the upper and lower wing surfaces. During the development in the pupal stage, it exists not as a pouch but as a flat area on the upper wing membrane which produces a copious amount of filamentous material. As the butterfly hatches and invagination occurs, this is incorporated within the pouch. According to Eltringham (1915), the cells which elaborate this material atrophy and are replaced by others which greatly enlarge and produce an oily secretion. This is discharged into the pouch and is taken up by the filamentous material. Thus in *Tirumala,* it appears that the wing glands produce a form of dust which has to be transferred to the hairpencils, while in *Amauris* the dust is produced in the hairpencil organ *per se.* Nevertheless, the effect must be the same in these two genera: the dispersal of scent around the female is aided mechanically by its adherence to the dust.

More evidence is needed for the wing glands of the Euploeini, but as far as is known they do not produce any kind of dust, although they appear to be secretory (Eltringham, 1915, 1935; Poulton, 1927).

### (E). *Sexual Scents of Male Danainae*

It is of the utmost importance to emphasize, as Fritz Müller (1877c, 1878) did nearly ninety years ago, that the scents of butterflies can arise from two different chemical systems: one related to protection against predators, and the other to sexual behavior. Characteristically, in those species which are unpalatable to predators (Brower & Brower, 1964) both sexes often have a common repulsive scent, though in the female it may be stronger. In contrast, the sexual scents are usually limited to the males, and as Dixey (1905, 1906a, b), Poulton (1906, 1907, 1927, 1929), Longstaff (1908), Eltringham (1925a), Clark (1926, 1927), Pycraft (1939) and Ford (1962) have pointed out for butterflies in general, these are often agreeable to man. This is a very important fact, but also is dangerously tautological because there is no *a priori* reason

why chemical releasers of sexual behavior in butterflies should smell pleasant to a member of the Phylum Chordata.

Both repulsive and sexual scents may occur in the same individual. Since the latter are often produced in areas which are in some way sheltered from the air (Müller, 1877a, b, c, 1878; Barth, 1959), they can easily be overlooked. Unfortunately, many of the naturalists upon whose observations the historical findings are based did not consider these facts. Moreover, the descriptions have had to be taken from individual opinions which are qualitative and subjective; for example, what appears pleasant to one person may not appeal to another. The advent of gas chromatography has now provided a means of quantitative comparison of even minute amounts, but has not yet been used to analyze butterfly scents, although it has for moths (Rothschild, 1960). Hopefully, chromatographic work in progress by L. Brower, T. Eisner, J. Meinwald and T. Pliske will shed much new light on this fascinating subject.

### 1. *The Lycoreini*

Müller (1878) described the hairpencils of *Lycorea* sp. and *Ituna ilione* as smelling strong and rather disagreeable. Longstaff (1914) stated that the hairpencils of two *"Lycorea atergatis"* individuals did not smell at all, and that those of a third smelled slightly like a cockroach. He indicated that his specimens came from either Trinidad, W.I., or Venezuela, which suggests that he was probably dealing with *Lycorea ceres ceres.* This is the only lycoreine in Trinidad (Kaye, 1921). Our research group has collected hundreds of males of this species in Trinidad and we are of the opinion that the scent of the hairpencils is flowery, but at the same time musky; curiously, men, more often than women, consider it pleasant. Since these butterflies do not possess the wing glands characteristic of the other two tribes of the subfamily, there can be little doubt that the hairpencils both secrete and disseminate the scent.

### 2. *The Euploeini*

Longstaff (1905, 1908) stated that various species of euploeines (and danaines) smelled of acetylene, but did not definitely determine the source of the smell. He later (*in* Lamborn, Longstaff & Poulton, 1911), thought that neither the hairpencils nor the wing glands produced it. Dixey (1906), citing Wood-Mason, said that the hairpencils of *Euploea radamanthus* Fab. are finely vanilla-scented. Longstaff (1912) extended his review of euploeines and cited an observation by Shelford that the hairpencils of

*Trepsichrois mulciber* are sweetly scented, as were those of *Euploea montana* Feld. Poulton, Lever & Simmonds (1931) mentioned that the hairpencils of Euploeas from Fiji smelled like burnt gingerbread or caramelized toffee. As already mentioned, Eltringham's (1915, 1935) histological studies of *Trepsichrois mulciber, Tronga brookei, Hestia lynceus* and *Euploea core asela* led him to believe that the hairpencils produce the scent in euploeines. There is thus no evidence that the wing glands produce scents in the euploeines, although in some species, as noted above (section IV-D), they appear to be actively secretory.

### 3. *The Danaini*

It is in this tribe that the greatest confusion has resulted from failure to distinguish between the protective and sexual scents. The major exception to this statement is the work of Müller. In his classical papers he reasoned by analogy with other butterflies that the wing pockets of the South American Monarch and Queen produce the scent, which, however, he was unable to detect on the wings of these butterflies. Müller explained this apparent contradiction by saying that the wing pocket was constructed to conserve the scent and would only give it up to the hairpencils when they were inserted through the narrow slit of the pocket. This appeared to him a satisfactory explanation of the characteristic scent of the hairpencils of these two species, which he described as similar to but less intense than the *Lycorea* hairpencils.

In our investigations of the Trinidad subspecies of the Queen butterfly (Brower & Jones, 1965), we have smelled numerous wing pockets of reared and wild males of a variety of ages and in no instance were we able to detect a scent arising from the intact wing pocket. On the other hand, the hairpencils of reared males in which the wing pockets had been sealed a few minutes after emergence did develop a sweet scent, thus proving that the hairpencils are independent secretory organs.

We also disagree with Müller's (1878) description of the scent as being unpleasant, but agree that it is less intense but broadly similar to that of the lycoreines. Elusive differences detectable to us exist in the Florida, Jamaican and Trinidad subspecies. This is of great evolutionary interest and we hope to explore the problem further.

Although Longstaff's papers (1905, 1908) mentioned that various danaines smell of cockroach, acetylene, muskrat, rabbit hutch, musty dung, stale tobacco smoke, slightly pleasant, sweet, *etc.*, the results are inconclusive with respect to which part of the butterfly they are emanating from, as he himself later pointed out (*in* Lamborn, Longstaff & Poulton, 1911).

Clark (1926, 1927) described the odor of the female Monarch butterfly as rather strong and disagreeable, resembling that of cockroaches or carrots. The males have the same odor but in them it is faint and overlaid with a very sweet odor like that of milkweed or red clover flowers. Apparently Clark thought that this was the sexual scent which emanated from the wing pocket, as he cited Scudder's (1889) statement that the scales of the pocket emit a slightly honeyed odor distinct from the carroty smell which all the scales possess. Hausman (1951) seems to have accepted this uncritically and did not mention the hairpencils at all. Urquhart stated that the hairpencils of the Monarch smell like the flowers of *Spiraea*. He also noticed a faint fragrant scent on the wings of the male.

Finally, Lamborn (*in* Lamborn & Poulton, 1918) described the scent of the hairpencils of *Amauris niavius dominicanus* Trimen in the wild as similar to aromatic snuff.

Considerable evidence points to the conclusion that the hairpencils of the three tribes of the Danainae produce a more or less fragrant scent, and that the wing glands of the Euploeini and Danaini either do not have any scent or, if they do, it is indistinguishable to the human nose from one of the component scents generally distributed over the wings or body of the butterfly.

### (F). *Mechanical Interaction of the Hairpencils and Wing Glands*

Although Müller (1877) originally argued that the position of the wing glands is such that the male could easily apply the hairpencils to them, the actual behavior was not recorded until 1911 when Lamborn (*in* Lamborn, Longstaff & Poulton, 1911) observed it in *Amauris niavius* and then again in *Amauris egialea* (*in* Lamborn, Dixey & Poulton, 1912). Having noticed a male settle on a leaf with its wings expanded, Lamborn then saw the insect arch its abdomen dorsally. This resulted in bringing the posterior of the abdomen to the level of the wing patches. Following this, the male extruded the hairpencils and by alternately flexing and straightening out its abdomen, passed them back and forth over the surfaces of the respective right and left wing patches. In another instance (Lamborn, *in* Lamborn & Poulton, 1913), the male *A. egialea* snapped its wings together each time it completed a cycle of brushing the hairs across the patches. Further details were observed in *A. niavius dominicanus* (Lamborn, *in* Lamborn & Poulton, 1918): the stiff dark hairs were spread

out fan-wise over the patch, while the light, softer hairs were applied without fanning out. By snapping the wings 10 to 12 times in 5 to 6 seconds, the male was able to move the stiff hairs over the patch while keeping the softer pencil stationary. These observations confirmed Eltringham's (1913) suggestion that the stiff hairs probably function to lift up the covering scales of the patch to facilitate secretion interchange with the rest of the hairpencil. In one example, the duration of this behavior from the time the male first extruded the hairpencils until it completely retracted them was 1 minute and 40 seconds.

The only other danaine in which the mechanical relationship has been observed is *Parantica agleoides* Moore. Lamborn (1921) stated that a male alighted on a plant, partially closed its hindwings, curved its abdomen dorsally and rubbed the protruded but unexpanded hairpencils over the patches at the rate of about 20 strokes per minute. This behavior occurred for nearly five minutes, after which he captured the insect. The position of the wings of *Parantica* during this behavior is thus similar to that proposed by Müller for *Danaus*. Urquhart (1958) was of the opinion that the Monarch males juxtapose the two organs, but gave no direct evidence other than that he once observed a male sitting on a leaf with its wings partially closed and the abdomen raised and moving in a somewhat jerky way from side to side. Stride (1958a) attempted to study the interaction in *L. chrysippus* by putting a slow-drying ink into the wing pocket, but was unsuccessful.

In the present investigation of *D. gilippus berenice*, only two observations were made. One male was seen sitting on herbage with its wings closed dorsally. Owing to a small hole in the right hindwing it was possible to see that it had bent its abdomen dorsally and was attempting to push the right pencil into the pocket. Instead it succeeded only in protruding it through the hole in the wing. The other observation was made inadvertently during the filming of an unsuccessful attempt to copulate. The male paused and raised its abdomen towards the right pocket. The hairpencil was not, however, extruded at this time. It is probable that the males insert their hairpencils into their wing pockets at frequent intervals while they are not engaged in courtship.[4] However, in our opinion, its occurrence during the unsuccessful courtship was significant only as a displacement activity. The fact that we observed it during actual courtship only once in the entire investigation strongly suggests that it is not a normal component of the interaction of the male and female while they are together.

For the Euploeini, even less evidence is available. According to Latter & Eltringham (1935), males of *Euploea core asela* have been seen applying the hairpencils to the scent area on the forewing, but the details of the behavior are unknown.

Thus the hairpencils seem to be the source of the scent, but are applied by the males to the wing glands. The possible functions of the behavioral interaction of these two glands will be considered in the discussion after the use of the hairpencils in the courtship has been described.

## V. METHODS AND MATERIALS

### (A). *Location and Ecology of the Study Area*

The experiments were conducted during the summers of 1960 and 1961 in the vicinity of the Archbold Biological Station in Highlands County, south central Florida. During parts of the Pleistocene Epoch when the sea level was higher than at present, Florida was considerably restricted in size, and the rolling sand-dune ridges which now characterize the central highlands were formed. (Deevey, 1949; Flint, 1957). Because of the topography and sandy soil, the area is well drained and supports a specialized scrub flora characterized by pines, palmetto palms and numerous species of bushy xerophytic trees (Davis, 1943). Among the herbs that grow within this community are various species of *Asclepias* (milkweeds) of the family Asclepiadaceae. These serve as larval foodplants of the Queen butterfly (Brower, 1961, 1962). The area has proved commercially valuable for the growth of citrus fruit and at the time of the study several new orange groves were being developed. The harrowing operations employed in clearing the land cut and spread the large tuberous roots of one of the milkweed species, *Asclepias tuberosa rolfsii* (Britton) Woodson. Consequently the plant was temporarily abundant and supported a substantial population of Queens.

The study area was specifically located at Childs Station. Once inhabited, this now deserted railroad depot is an old field surrounded by the new orange groves. Growing among the grass and weeds were a few live oak trees, Australian pines, and several overgrown, feral cultivated shrubs including *Mango* and *Hibiscus*. This presented an ideal situation for releasing female Queens, because in the afternoon males tended to fly in from the surrounding groves and congregate in quantities sufficient for continuous experimentation. Fortunately, their numbers were low enough so that a courtship by one male was not often interrupted by others.

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[4]This has now been confirmed for the Trinidad subspecies (Brower & Jones, 1965).

Preliminary work indicated that the courting activity of this species is concentrated in the afternoon so that it was decided to experiment between 2 and 5 p.m. Eastern Standard Time. The methods were worked out in June and July of 1960, and the experiments were conducted between July 22 and August 11 in 1960 and between July 2 and August 11 in 1961. Throughout both summers laboratory-reared females were presented to wild males in their natural environment according to the method described below.

### (B). *Rearing of the Females*

To obtain eggs for rearing quantities of butterflies, wild females were caught in the field and temporarily stored in glassine envelopes (Ward's Natural Science Establishment, Inc.). When a sufficient number had been captured, one to four were released inside oviposition cages which had been placed over blcssoming young milkweed plants *(A. t. rolfsii)*. The cages were one-foot cubical structures consisting of a wooden frame with plastic screening on the top and the three sides and with plywood on the fourth to provide shade during part of the day. During 1960 the females were fed daily on a honey-water solution (approximately 1 part clover honey to 4 parts water) to supplement the nectar, but in 1961 this was not done because plants with sufficient flowers were located and a maximum of two butterflies was released in each cage. At intervals of 24 hours the eggs were collected with forceps by plucking off the leaves and individual florets on which they had been laid. These were put into containers to prevent desiccation and taken back to the laboratory where the larvae hatched and were fed on *A. t. rolfsii*.

This foodplant was collected each day in the orange groves or in recently cleared scrub areas by breaking off the stems of young healthy plants and accumulating them in plastic bags. It was important to keep these bags shaded at all times to prevent subsequent rapid deterioration of the leaves. In the laboratory leaf-bearing stalks were dipped in water to wash off the sand which was splashed up on them by the heavy rainfall that occurs in the region. After shaking off the water, they were spread out on paper and allowed to dry off, but not wilt. They were then placed in an inch of water in quart plastic containers, covered with a plastic bag and stored in a refrigerator until used later that day or early the next. At the time of their use, they were broken into short pieces still bearing their leaves, and placed in the rearing containers. The leaves on the basal part of the stalk deteriorated while in the water and care was taken not to feed them to the larvae.

During 1960, the rearing was done in the laboratory, at first under variable conditions but later in a controlled environment room which proved highly advantageous. In this room eggs were placed singly (by twos in 1961) in half-pint containers with two or three fresh *A. t. rolfsii* leaves. These containers are made of clear plastic and are manufactured by the Wilpet Tool and Manufacturing Company, Kearny, New Jersey (Wilpak VSH No. 208). The internal measurements are: height 4.7 cm., width at the top 8.9 cm. and width at the bottom approximately 7.5 cm. They have snap-on caps which have two right angle air vents approximately 0.2 cm. wide and .05 cm. deep. To help prevent desiccation of the leaves and eggs in the container, two damp cotton wads were added. These were about one-half inch in diameter and had been immersed in water and then squeezed out. All containers were placed on a table approximately 36 inches below a bank of G.E. 8-foot "slim-line" fluorescent lights, balanced with 18 artificial and 6 daylight tubes so as to simulate sunlight. The surface area of the table corresponded to that of the bank of lights and was approximately 96 by 48 inches. The lights were connected to a time switch which turned them off at 8 p.m. and on at 8 a.m. in 1960, so that each day consisted of a 12-hour light and a 12-hour dark period. In 1961, they were set to come on an hour earlier with a 13-hour light and 11-hour dark period. In 1960, one large group of Queens was reared at 25°C (77°F) and another at 30°C (86°F). In 1961, all were reared at 28.3°C (83°F). Short term fluctuations of temperature occurred to the extent of $\pm$ 2°F and at rare intervals rose for short periods (less than 1 hour) as much as 5°F due to factors beyond our control. A fan circulated air beneath the light bank during the light period. At 25°C the mean development time from oviposition to emergence of the adult was 24.3 days, ranging from 22.8 to 26.9 days $\pm$ 3 hours. At 30°C corresponding rates were a mean of 18.5 days, ranging from 16.8 to 19.9 days $\pm$ 2 hours (Brower, ms. in preparation). In 1961 the precise time of development was not measured, but averaged slightly under three weeks.

Between two and three days after oviposition the eggs hatched. All containers were checked once to twice daily, depending on the stage of larval development. Towards the end of the fourth instar, the larvae were isolated so as to have one per container. The fifth instar larvae ate voraciously and so required more frequent care than the younger ones. Fresh milkweed leaves were added daily, uneaten ones and fecal pellets were cleaned out and moisture which had condensed on the inner sides of the containers was

wiped out with a Kleenex tissue. The larvae were not handled or removed from a leaf or stalk since this causes high mortality. Instead, if they were on a leaf, this was picked up, cut to minimal size and replaced with the fresh food. The larvae completed their development in about two weeks and metamorphosed to chrysalids which hatched approximately one week later. In 1960 the larvae formed their chrysalids on the tops of the containers but this proved unsatisfactory since the emerging butterflies became crippled as they attempted to expand their wings within the limited space. Therefore in 1961 larvae about to pupate were transferred in the early afternoon to a 2-foot cubical cage constructed of plywood with a screen back and a hinged front screen door. Their containers were opened and an excess of food was added. Within a few hours they crawled to the top of the cage and began to spin up as described in detail by Urquhart (1960) for the Monarch butterfly.

During the course of rearing the animals in the controlled environment room, it was found that changes from instar to instar, from larvae to pupae, and from pupae to adults assumed a 24-hour periodicity rhythm and preparation for the changes always began late in the afternoon. All adults hatched within one to two hours of the artificial "dawn." It thus appears that the Queen butterfly has a circadian rhythm which synchronizes development stages. Such "24-hour clocks" are well known in other animals (Pittendrigh & Bruce, 1959; Beck, 1964).

The advantages of rearing under the controlled conditions cannot be overemphasized. Mortality due to disease, failure to develop properly or accidents in handling were less than 25% from egg to adult. No epidemic diseases occurred at all, whereas under variable room conditions and less rigorous cleaning and feeding schedules, mortality often was over 80%. The synchrony in development obtained under the controlled conditions is partly responsible for this, since it enables one to avoid manipulation at the critical time of change between successive stages in development. In 1961, by setting up approximately two dozen eggs at 24-hour intervals, it was possible to produce a daily supply of freshly emerging adults.

### (C). Isolation and Aging of the Females

Late in the morning after the butterflies had hatched, the females were transferred from the laboratory to outdoor cages (approximately 8 feet by 8 feet by 7 feet high) where they were kept in isolation from males but not from each other. During the summer of 1960, females of ages varying from 1 day to 10 days post-

hatching were used. Their mating history prior to a particular experimental courtship ranged from virgin to four times previously mated, though none was inseminated. The number of times each underwent courtship varied from once to 26 times over the ten-day span. In 1961 we extended the study to investigate the role of visual selection by males when presented with females of modified color pattern. Since we were interested only in the effects of color, the procedure was standardized to eliminate the variables of age, number of times mated and number of times courted. All females were used only on the afternoon of the second day after they hatched and were released to males until mated or until they had undergone a maximum of three courtships without mating. In this way a female which had been mated was not used again. Two-day-old virgins were used for two reasons. First, younger ones were apt to be insufficiently hardened after emergence and therefore difficult to handle without damage. Secondly, females older than two days tended to fly out of the range of easy recapture and often became lost after their first release. In this paper, the 1961 findings are limited to the experiments with females which were not altered in color and served as normal controls in the color modification experiments. The results of the latter study have been summarized elsewhere (Brower, 1963).

The butterflies were kept in the outdoor cages and when they were to be used in the courtship experiments were caught by hand and then put into glassine envelopes and conveyed to the field in a cylindrical 1.5 quart plastic container. In 1960 they were returned to the cages after their use even if they had been mated, so that virgin and non-virgin females were kept together. However, in 1961 virgins were always kept separately. During both summers the reared males were not used except in some preliminary experiments discussed below.

In 1960 the caged females were provided with *A. t. rolfsii* flowers as a source of liquid food. The stalks bearing the flowers were stripped of their leaves and put into 8-ounce Coca-cola bottles full of water which were wired at varying heights from the ground to all sides of the cages. The butterflies fed freely, but because the flowers tended to senesce rapidly, it was necessary to supplement them with honey solution. In 1961, milkweed flowers were in short supply so the butterflies were fed only the honey-water solution.

At the time of release into the isolation cages, each female was numbered with black "magic-marker" (Cado Permanent marker, Esterbrook Pen Co., Camden, New Jersey) so that it could

be individually recorded throughout its use in the experiments.

### (D). *Presentation of the Females to the Wild Males*

At the beginning of the study in 1960 several different ways of presenting the females to the males were attempted. They were first tied with thread as described by Brower (1958) and the thread attached to a stick as Tinbergen *et al.* (1942) had done in studying the Grayling butterfly. In this way the female could fly at the end of its tether and be presented to any passing male. However, few sustained responses were obtained, and the aerial component of the courtship observed under natural conditions was not adequately expressed.

In a second attempt we put several wild-caught males into outdoor cages similar in construction to those already described but considerably larger. Females were then released by hand to these males which did court and in some instances succeed in copulating with them. However, the aerial component was again restricted as the females flew towards the sides of the cage. Moreover, because the males interfered with each other, insurmountable problems of interpretation resulted. An additional problem, similar to the one described by Stride (1958a) in his cage studies of *Limnas chrysippus,* was that the males, although they responded to the females initially, soon courted them with lowered intensity and eventually ceased to exhibit sexual behavior altogether.

We therefore returned to the field and tried a new method of releasing females singly to the wild males in their natural environment. Our reason for not doing this before was based on the knowledge common to all who have caught butterflies: when released they almost invariably fly off in a rapid escape flight. However, it was discovered that the male could easily fly after, overtake, court and often successfully mate with her. This technique was therefore standardized according to the following procedure: the female was removed from her storage envelope and held gently at the junction of wings and thorax between thumb and forefinger so that her wings folded naturally over her dorsum. She was then carried around in the courtship area in this way until a male was found resting on or flying over the herbage or feeding at flowers (usually the composite, *Bidens pilosa* Linnaeus). Once located, the male became the "target" to which the female would be thrown and it was necessary to approach him carefully so as not to stimulate his escape flight. If the male were feeding or resting, we usually waited until he flew up or was about to do so. The female was then gently launched so that she would fly past his anterior. This was successful in nearly all instances. Occasionally, however, a female did not fly when released but instead plummetted to the ground. Such releases were disqualified, as were those in which the male either did not see the female or did not respond to her. When the attempt failed for any of these reasons or when the courtships terminated and the female began to fly away, she was caught in a butterfly net, brought back to the center of the area and launched again. To make this procedure more uniform, one of us served as launcher and the other as netter.

Although wild males were present in the courtship area at nearly all times, their numbers were not sufficient either to allow the release of each female to a different male, or to remove males from the area after they had mated with our females. However, we did mark the males before we released them back into the natural population. Consequently, it was always possible to tell whether a particular male which had just mated with one of our females had mated with others before, but we could not know whether a courtship which failed to terminate in mating involved one of these males or a new one. Moreover, females were released to the same male on several occasions, both on the same and on different days. Thus all individual courtships, although each is considered as a single numerical unit, are not completely independent in the statistical sense. However, this lack of statistical independence is small both because few males mated with more than one experimental female and because the study in both years was carried out over many days during which there was a continual turnover of the males. Moreover, we always captured the pairs as soon as they had mated and gently separated them. As a consequence, the males did not have time to inseminate the females, which would have lowered their sexual drive considerably (Norris, 1932).

### (E). *Motion Picture Analysis*

It was not possible to take extensive motion picture sequences for quantitative analysis because of the near impossibility of maneuvering the camera close enough to the butterflies or keeping it in focus during an entire courtship. However, it was relatively easy to film individual phases of their sexual behavior. To do this a Bolex H-16 reflex camera (16 mm.) was used primarily with the Kern Paillard 25 mm. Switar lens, but also with the Som Berthiot Pan Cinor 25-100 mm. zoom lens, the K.P. 150 mm. Yvar telephoto lens and the K.P. 10 mm. Switar wide

angle lens. Ektachrome Commercial 7255 film was used and most sequences were taken at 24 frames per second and a few in slow motion at 64 frames per second. The battery-operated Bolex motor was used to film long scenes but it does not operate at high enough speeds to take slow motion scenes. A copy of the film made in 1960 was studied by single frame observation in an editor and by projection. This was useful in interpreting the behavior and suggested further work, which was completed in the summer of 1961. In addition we also made an 18-minute film which shows the courtship and the methods used in studying it. (Brower & Cranston, 1962). Copies of this may be rented or purchased from the Psychological Cinema Register, Pennsylvania State University, University Park, Pennsylvania, (P.C.R. film 2123K). Plate II, figures 3 and 4, were made from one behavioral sequence on the movie film. The individual color positive frames were projected through an enlarger onto a panchromatic black and white negative film, and these were subsequently enlarged to make black and white positive prints. (Plate II, figures 1-2, were taken with a Hasselblad 500C camera with an 80 mm. Zeiss Planar lens (Synchro Compur shutter) at 1/6000 of a second (Ascor 323 electronic flash) on Kodak Plus X Pan Professional black and white film.)

### (F). Tape Recording the Data

Early in the summer of 1960 an attempt was made to obtain quantitative results by having one person observe and announce the events while a second individual wrote them down. However, the courtship takes place so rapidly that this method proved inaccurate. Therefore a small portable tape recorder[5] was used to record verbally a precise description of each experimental courtship, thus permitting continuous observation of the behavior as it occurred. In the evening the recording was played back into another tape recorder and a second tape was made which served as a permanent record to be analyzed later in detail. The original tape was then erased and used for recording the next day's observations.

Because of the complexity of the courtship, one of us announced the male's behavior and the other the female's behavior. The fidelity of the recorder was such that the two voices, even if heard simultaneously, could easily be distinguished. Because of the small size of the recorder, it was possible to run after the courting but-

terflies and keep close to them at all times, unless they flew high into the air or into a tree, which infrequently happened. Occasionally the pair would fly through a bush and evade us. In such instances, we would approach the bush from opposite sides so as not to lose sight of them. Even when this happened, the sensitivity of the machine was still sufficient to record both voices. Our voices did not appear to affect the butterflies during their sexual activities, although it was necessary to be careful not to make quick movements or to cast shadows on them.

Quantitative data were obtained by transcribing the verbal sequences to written ones and then tabulating the frequency and duration of each of the components in all courtships. Duration was determined with a stopwatch to the nearest second. The methods of transcribing and duration measurements are easily repeatable by two people independently. The permanent tapes were also used for rechecking the data and proved exceedingly valuable for reference. For example, in the course of the 1961 field work, new hypotheses arose which were supported with data by referring to the 1960 tapes.

### (G). Numbers of Individuals Studied

In 1960, 187 courtships of 40 different females were recorded and analyzed. These females varied in age, mating history and the number of times courted, as described above. During 1961, 79 courtships of 41 two-day-old virgin females were studied, making a total of 266 courtships of 81 females. As will be seen, even though the 1960 females were heterogeneous compared to those in 1961, the findings for the two years, with minor exceptions to be discussed below, are similar in nearly all respects. This is most important because the hypotheses formulated on the basis of the 1960 data were confirmed by the experiments in the second year.

The first section of the results will present the descriptive aspects of the courtship, and the quantitative comparisons will be considered in the second part. The description was also partly formulated from observations made during the color-modification experiments in 1961, so that a total of over 325 courtships of more than 125 females has been considered in arriving at the conclusions presented in this paper.

### VI. Results

The sexual behavior of the Queen butterfly is a complex sequence of interactions of the male and female. These occur in four main components and consist of nine different phases (Table 1). This study will consider the first three components of the courtship from the initial pursuit

TABLE 1. COMPONENTS AND PHASES OF SEXUAL
BEHAVIOR IN THE QUEEN BUTTERFLY

I. First Aerial Component
    Phase 1. Aerial pursuit
    Phase 2. Aerial hairpencilling

II. First Ground Component
    Phase 3. Ground hairpencilling
    Phase 4. Hovering and striking
    Phase 5. Copulation attempt
    Phase 6. Copulation

III. Second Aerial Component
    Phase 7. Post-nuptial flight

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IV. Second Ground Component
    Phase 8. Insemination
    Phase 9. Termination of copulation



TEXT-FIG. 1. *Aerial hairpencilling, phase 2.* The male is above the female and his hairpencils are shown partially splayed. Approximately .75 natural size.

(phase 1) through copulation (phase 6) to the beginning of the post-nuptial flight (phase 7). The fourth component which includes two phases, insemination and termination of copulation, was not analyzed in detail. During courtship a male often repeated the various phases leading to copulation and a considerable number of courtships consisted of two or more first aerial components. These proved extremely important in elucidating the behavior and will be called *multiple first aerial component courtships.*

### (A). *Description of the Courtship Behavior*

### 1. *Successful Courtship*

When the female appeared in the visual field of the male, he flew after her in an *aerial pursuit* (phase 1). She continued to fly and might accelerate, but the male is capable of flying faster and rapidly overtook her in the air. As he passed a few inches over her dorsum, his mode of flight changed suddenly to a rapid bobbing, and his extruded abdominal hairpencils were rapidly swept up and down over her head and antennae. During this *aerial hairpencilling* (phase 2, text-figure 1; Plate III, figure 1), the male maintained his forward flight motion and though he often buffeted the female, he always stayed in front of her. The hairpencilling apparently serves to disseminate the perfume over the female's antennae as she flies through the air and may also act as a tactile stimulus. She responded to this activity by slowing her forward motion and by descending towards the ground where she alighted on available herbage.

As she landed, the courtship entered the first phase of the ground component, known as *ground hairpencilling* (phase 3, text-figure 2; Plate II, figures 1-4). In this, the male continued to hairpencil and buffet with the same bobbing
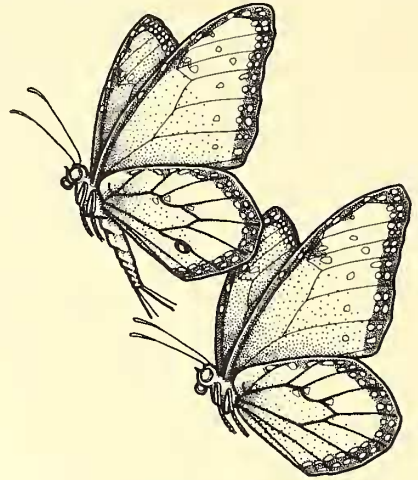
flight motion as before, except that he maintained a position close to her anterior without moving forward. This phase was often particularly intense as he rapidly danced in front of her. A second directional component might be added when he shifted from up and down to lateral bobbing in which he swept his hairpencils back and forth in front of her through a semicircular arc up to about six inches in diameter.

The response of the female to the hairpencilling male was either to fold her wings tightly over her back or to flutter them while still holding on to the herbage. When the latter occurred,
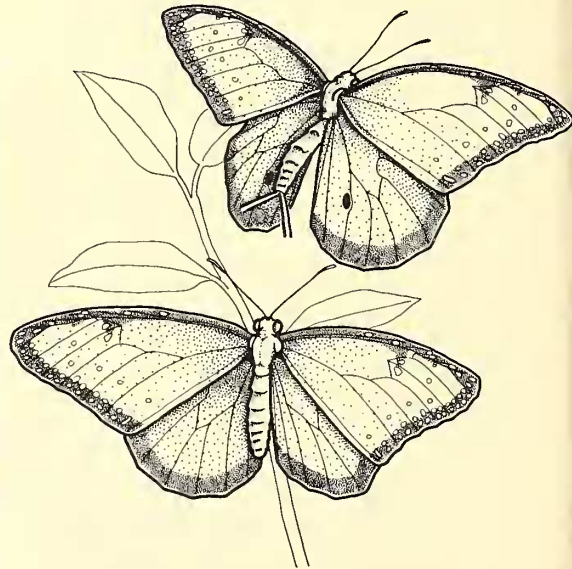


TEXT-FIG. 2. *Ground hairpencilling, phase 3.*
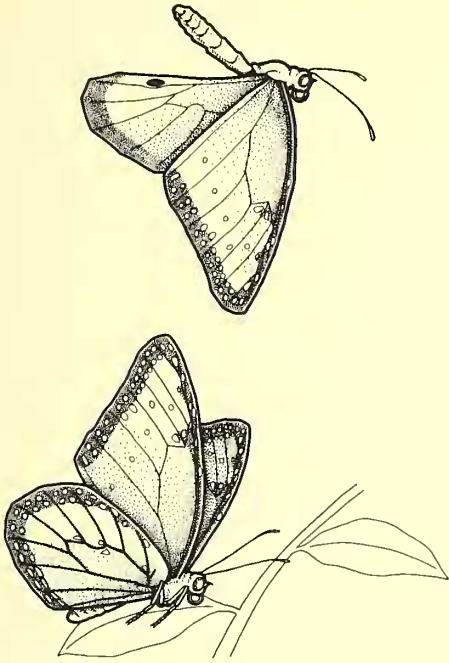
TEXT-FIG. 3. *Hovering and striking, phase 4.* The male is shown at the moment of the downward beat of his wings as he hovers above and in front of the female.

the male usually retracted his hairpencils, hovered a few inches above her, and intermittently dropped momentarily onto her dorsum. This phase of the courtship is called *hovering and striking* (phase 4, text-figure 3; Plate III, figure 2), was extremely variable in duration, and was short or even absent when the female was immediately receptive; more will be said about it below.

The folding of the wings by the female represents a positive reaction to the male's hair-

pencilling and probably serves to stimulate his next response which is to retract the hairpencils and alight laterally to *attempt copulation* (phase 5, text-figure 4; Plate III, figure 3). He nearly always alighted in the same direction that she was facing by rapidly dropping from the hovering position on to her right or left side. As he alighted, he grasped the undersides of the fore- and hindwings of the female with his meso- and metathoracic legs in a position such that the two butterflies formed an angle with their parallel bodies at its base and their respective appressed forewing tips at its apices. This angle varied roughly from 45° to 90°. The male sometimes helped to balance himself by intermittently fluttering or by holding on to the herbage in addition to the female's wings. Shortly after alighting on the female, the male twisted his abdomen laterally and probed the lower area of her hindwing in an attempt to make contact with the tip of her abdomen. By this time he had extruded his clasping organs and eventually thrust his abdomen up between her hindwings, located her genitalia and attached his claspers thereto. He sometimes poked the undersides of the female's hindwing several times over most of its area before he succeeded in contacting her genitalia. It was not possible to observe the genital contact because the tips of their bodies were hidden inside the female's hindwings. (This difficulty could be overcome by cutting a small hole in the wing of the female). The agility with which the male maneuvered his abdomen is remarkable and shows that it is a highly specialized prehensile organ. Throughout the entire copulation attempt, the female kept her wings folded over her dorsum and appeared to remain passively quiescent. Both during and after the copulation attempt, the male palpated the antennae and dorsal head region of the female by alternate movements of his right and left antenna (text-figure 5).



TEXT-FIG. 4. *Lateral copulation attempt, phase 5.* The male is holding onto the undersurface of the female's left wings and is twisting his abdomen towards hers.
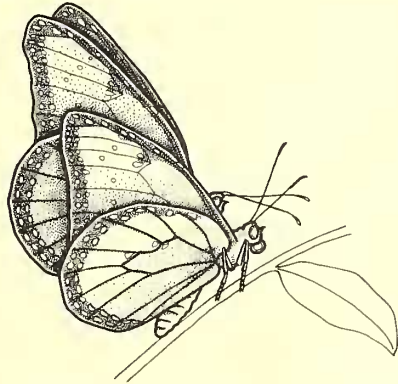


TEXT-FIG. 5. *Antennal palpation* by the male as the female clings to herbage.
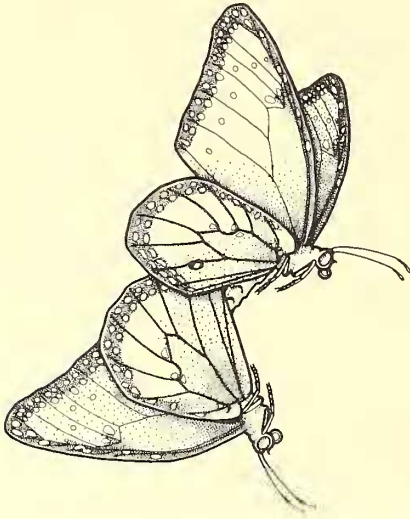
TEXT-FIG. 6. *Post-nuptial flight, phase 7*. The male flies off carrying the quiescent female *in copulo*.

Shortly after *copulation* was achieved (phase 6), the male opened his wings slowly to an angle of about 60° and then quickly closed them. This *wing-snapping* might be repeated but usually occurred only once. It was invariably followed by the *post-nuptial flight* (phase 7, text-figure 6) which commenced as the male flew off carrying the female suspended at the end of his abdomen. As she was carried away upside down, she neither struggled nor attempted to fly, but kept her wings appressed and her legs folded against her thorax. In no instance did the female carry the male. The post-nuptial flight varied in height and distance and was sometimes spectacular. For example, when pursued by us, or by a second Queen male, the male sometimes carried the female as high as 80 feet and for a distance of well over 1,000 feet. The flight ended as he settled with her inconspicuously among ground herbage or in a bush or tree (Plate IV). If the pair was disturbed, the male reinitiated this phase of their behavior. It seems most likely that the function of the post-nuptial flight is to carry the pair away from where they have been so conspicuously active to a less obvious area and thereby to reduce predation from vertebrate enemies. *Insemination* (phase 8) occurs after the pair has settled, and is followed by *termination of copulation* (phase 9) from about one to several hours later. These two phases were not analyzed in the present study.

### 2. *Unsuccessful Courtship*

The courtship can end during any of the first five phases of the behavioral sequence, and active termination by both sexes occurs (Table 2). A male would often pursue a female until he caught up with her and then break away without aerial hairpencilling. Other courtships progressed into the aerial hairpencilling phase and then ended as the male flew away. Since these courtships were unsuccessful because the male actively left the female during either phase 1 or phase 2, they were termed *aerial dismissal*.

In other courtships the females gave definite negative responses to the males in either of these two phases. The first of these was *aerial evasion* in which she kept flying and would not respond to him by alighting. Her behavior included rapid climbing and veering as well as hard downward and straight line flight. Occasionally the pair flew high into the air over distances comparable to the post-nuptial flight before the male would finally leave her and fly toward the ground.

TABLE 2. SIX CATEGORIES OF UNSUCCESSFUL COURTSHIP

| Termination by the Male | Component of Occurrence | 1960 | 1961 | Totals |
|---|---|---|---|---|
| A. Aerial dismissal | I | 43 | 7 | 50 (27%) |
| B. Desertion* | II | 38 | 7 | 45 (24%) |
| C. Homocourtship | II | 13 | 3 | 16 (8%) |
| Subtotals | | 94 | 17 | 111 (59%) |
| Termination by the Female | | | | |
| D. Aerial evasion | I | 9 | 2 | 11 (6%) |
| E. Rejection* | II | 22 | 25 | 47 (25%) |
| F. Foliage evasion | I | 11 | 8 | 19 (10%) |
| Subtotals | | 42 | 35 | 77 (41%) |
| Grand Totals | | 136 | 52 | 188 |

*Arbitrarily determined as Desertion or Rejection if the ground component of the courtship lasted, respectively, ≤ 20 seconds, or > 20 seconds.

A second way in which the female could avoid the male was by *foliage evasion.* Sometimes she would fly directly through a bush or tree and the male would lose sight of her and search for a few moments where she had entered. At other times, she would fly into the foliage and immediately alight, close her wings over her back, and remain motionless. Again the male would search but fail to find her. This behavior indicates that the male is oriented primarily by sight during the aerial pursuit phase of the courtship.

The female could also terminate the courtship after being induced to alight by not folding her wings dorsally in response to the male's hair-pencilling. *Rejection* by the female was arbitrarily defined as an unsuccessful courtship which lasted for more than 20 seconds in phases 3–5. It was in these courtships that phase 4, the hovering and striking behavior of the male, often became greatly prolonged. Instead of folding her wings, the female would alternately flutter and extend them laterally. The male would hover above her while she was fluttering and each time she stopped he would attempt to alight dorsally on her outspread wings. If he succeeded, she would usually flutter again and drive him off, but frequently he would attempt dorsal copulation (text-figure 7). The female without exception would prevent copulation from this position by fluttering him off, by thrusting her wings down in a single hard motion so that the male would lose his balance, and/or by vigorously twisting her abdomen away from the end of his. Occasionally after a long period of this activity, the male would succeed in alighting on the female laterally, the position from which copulation occurred. However, the female could still resist his attempt by similar evasive movements of her wings and abdomen (text-figure 8).

These sustained periods of courtship often came to an end as the male began to add a lateral
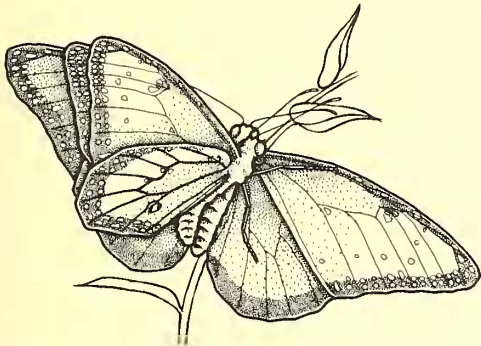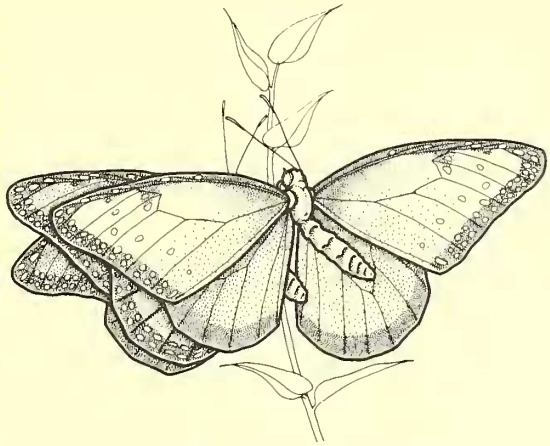


TEXT-FIG. 8. *Evasive behavior of female* during a lateral copulation attempt. The male is shown beneath the left wings of the female posturing his abdomen towards hers. Simultaneously she bends her body dorsally and thrusts her wings ventrally, all the while clinging to the herbage.

component to his hovering flight, which gradually increased until he hovered more widely in front of her and finally flew away. Her behavior influenced his time of departure. If she closed her wings as he hovered away and kept them closed, he would leave her sooner than if she fluttered them again as he hovered back towards her. It has already been seen that the wing-folding is a positive stimulus to the male if he is close to the female, but it also appeared to be a neutral stimulus if he was far enough away: by closing her wings she became inconspicuous so that he could no longer see her.

Such long courtships frequently ended in *homocourtship.* This occurred when another or several other males were attracted by the visual stimulus presented by the courting pair. The first male would fly up towards the intruder which in turn would respond to him instead of to the female. The males would then often fly off together leaving the female behind. The probability of courtship ending in this way depended both on its duration and on the numbers of males in the area. Thus the longer the courtship or the higher the population density, the greater were the chances of its terminating by homocourtship. This activity was always of short duration and soon ceased as the males flew their separate ways.

The male could also terminate the courtship after he had induced the female to alight by *desertion, i.e.* leaving the female after courting her for ≤ 20 seconds in phases 3–5 (Plate III, figure 4). Sometimes he would hover in front of her for only a short while and then fly away. At



TEXT-FIG. 7. *Dorsal copulation attempt.* The male (his wings folded) is shown holding onto the outspread wings of the female as he unsuccessfully attempts to mate with her.

other times, he would attempt to copulate either dorsally or laterally but without sustained vigor and then leave her.

These findings clearly show that both sexes have the power of rejection and can both terminate the courtship during any part of the behavioral sequence prior to copulation. Moreover,

they show that the density of the local male population is important in determining the outcome of the courtship.

3. *Multiple First Aerial Component Courtships*

Many of the successful courtships did not fol-

## COURTSHIP OF THE QUEEN BUTTERFLY

FEMALE BEHAVIOR              MALE BEHAVIOR

appears

pursues in air

flies

overtakes and hairpencils

alights on herbage

hairpencils while hovering

folds wings

alights laterally

acquiesces

copulates

post-nuptial flight
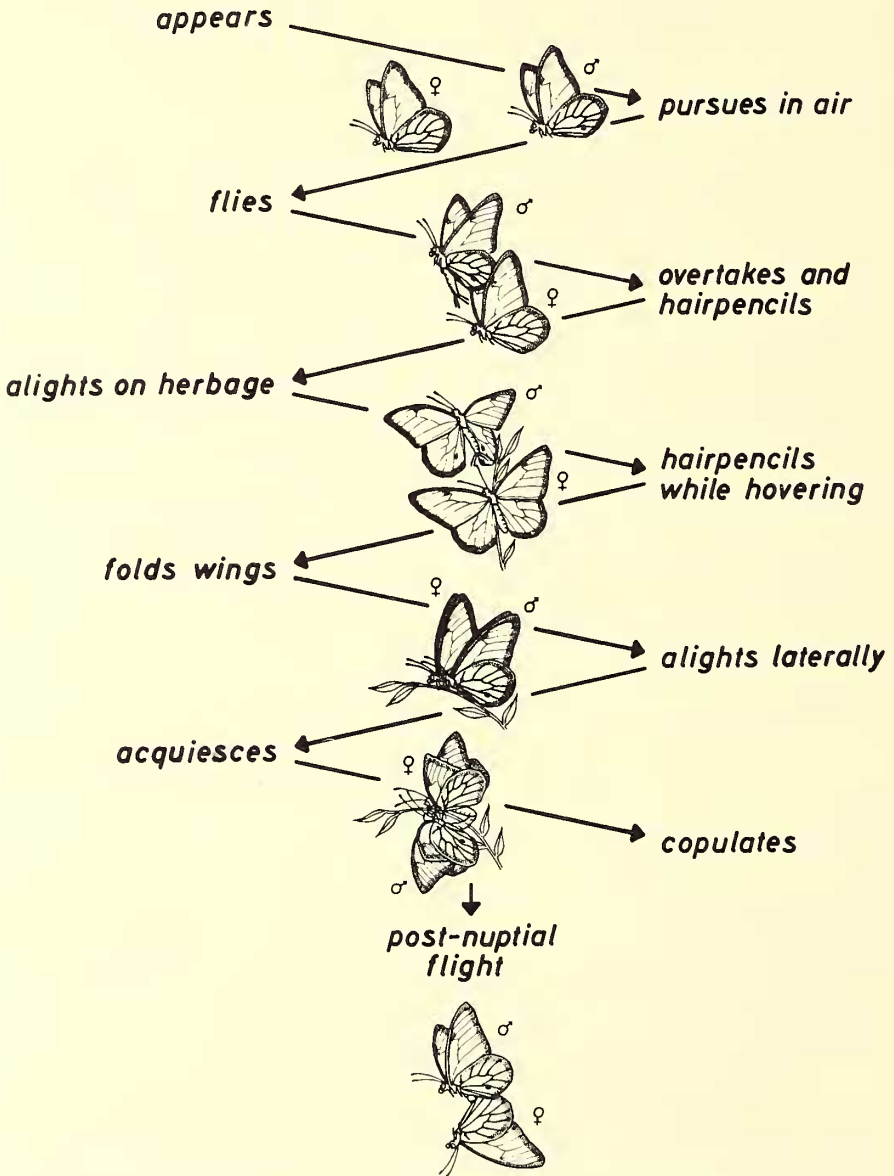
TEXT-FIG. 9. *Summary of the stimulus-response reaction chain in the courtship of the Queen butterfly.* The male behavior is shown on the right and the female behavior on the left.

low the step-by-step sequence of phases described above and summarized in Text-figure 9. Any of the first five phases could be repeated several times either alone or in various combinations (Table 3). For example, a courtship might proceed to the attempt to copulate (phase 5) and then revert to phase 4 as the male flew up from the female and hovered in front of her. In other courtships the hovering and striking (phase 4) might be followed by ground hairpencilling (phase 3), or the male might revert directly from attempting to copulate to ground hairpencilling (phase 5 to phase 3). Often the male would alternate between hovering and striking and attempting to copulate for long periods of time. Nearly always when this happened, the male neither hairpencilled again nor succeeded in mating unless the aerial pursuit flight was reinitiated. When this occurred, the courtship was termed a *multiple first aerial component courtship* and these more often ended in copulation than did those with a single aerial component (Table 4).

The greater chance of success in these multiple courtships was not apparent until after the statistical analysis of the 1960 data. This finding led to the discovery of the functional significance of the hovering and striking behavior of the male (phase 4) which had been so noticeable in the long courtships. In 1961, it became clear that the female would often be induced to fly up again by the hovering and striking of the male. As soon as she flew up, the male would pursue her and the courtship would start over again, often leading through all the phases to copulation. Thus the hovering and striking phase is of great importance, since a female which is at first unreceptive may eventually be mated if the male persists. Moreover, it leads directly to the possibility of sexual selection, because if two males have differential stimulating powers, the more persistent one will on the average be more successful in mating.

(B). *Quantitative Analysis of the Courtship Behavior*

The data tabulated from the tape records are

TABLE 3. Sequence and Repetition of the Phases in Successful Courtships in 1961

| Courtship No. | Phase sequence |
|---|---|
| | Successful Courtships with Single First Aerial Component (N = 12) |
| 24b<br>27a<br>185c<br>319a | 1, 2, 4, 3, 5, 6, 7 |
| 43a<br>59a<br>65a<br>67c<br>142a<br>221a<br>294a<br>314a | 1, 2, 3, 5, 6, 7 |
| | Successful Courtships with Multiple First Aerial Components (N = 15) |
| 44b<br>129a | 1, 2, 3, 2, 3, 5, 6, 7 |
| 330a | 1, 2, 4, 2, 3, 5, 6, 7 |
| 198a | 1, 2, 3, 5, 2, 4, 5, 6, 7 |
| 187a | 1, 2, 3, 4, 1, 2, 3, 5, 6, 7 |
| 191b | 1, 2, 4, 3, 4, 2, 3, 5, 6, 7 |
| 30a | 1, 2, 3, 2, 3, 5, 2, 3, 5, 6, 7 |
| 120a | 1, 2, 3, 4, 2, 3, 5, 3, 5, 6, 7 |
| 135a | 1, 2, 4, 3, 1, 2, 4, 3, 5, 6, 7 |
| 114a | 1, 2, 3, 4, 2, 3, 4, 2, 3, 4, 2, 3, 5, 6, 7 |
| 292a | 1, 2, 4, 3, 1, 2, 4, 5, 1, 2, 4, 3, 5, 6, 7 |
| 297c | 1, 2, 3, 5, 4, 2, 4, 5, 4, 5, 4, 2, 4, 5, 6, 7 |
| 116a | 1, 4, 1, 4, 1, 4, 1, 2, 3, 4, 2, 3, 4, 1, 2, 4, 2, 3, 4, 2, 3, 5, 6, 7 |
| 143c | 1, 2, 3, 4, 5, 4, 5, 4, 5, 4, 5, 4, 5, 4, 5, 4, 5, 4, 5, 4, 5, 4, 5, 4, 1, 2, 3, 5, 1, 3, 5, 6, 7 |
| 269a | 1, 2, 3, 4, 5, 4, 5, 4, 5, 4, 5, 4, 1, 2, 3, 4, 5, 4, 5, 4, 5, 4, 5, 4, 5, 4, 1, 2, 3, 2, 5, 6, 7 |

TABLE 4. RELATION OF SINGLE AND MULTIPLE FIRST AERIAL COMPONENT COURTSHIPS
TO THE SUCCESS OF COURTSHIP

| Outcome of Courtship | Category of Courtship | | | | | | Totals | | |
|---|---|---|---|---|---|---|---|---|---|
| | Single Aerial Component | | | Multiple Aerial Component | | | | | |
| | 1960 | 1961 | Total | 1960 | 1961 | Total | 1960 | 1961 | Total |
| Successful | 30 | 12 | 42 | 21 | 15 | 36 | 51 | 27 | 78 |
| Unsuccessful | 119 | 36 | 155 | 17 | 16 | 33 | 136 | 52 | 188 |
| Totals | 149 | 48 | 197 | 38 | 31 | 69 | 187 | 79 | 266 |
| % Successful | (20%) | (25%) | (21%) | (55%) | (48%) | (52%) | (27%) | (34%) | (29%) |

Significance: by inspection there is no significant difference between the 1960 and 1961 data, which are therefore lumped in a 2 × 2 contingency table. (Chi square = 23.6, d.f. = 1, P < .001).

presented in full for 1961 (Table 5) to enable verification of the analysis and to allow direct comparison of future studies by variance analysis. The various aspects of the data for both 1960 and 1961 as summarized in Tables 2, 4 and 6-9, show that the findings for the two years are in general very similar and justify the omission of the detailed records for 1960 due to lack of space. The analysis that follows will show (1) the extent to which the butterflies participate in the 7 phases of courtship, (2) the time spent in each of the first 6 phases and in the whole courtship, (3) the sequence and repetition patterns of the 7 phases, (4) the frequencies with which courtship terminates prior to copulation due to various causes and (5) the frequencies and significance of dorsal and lateral copulation attempts.

### 1. Participation in the Seven Phases of Courtship

Table 6 and text-figures 10a-c summarize the numbers and frequencies of participation in the seven phases of courtship by successful, unsuccessful and all courting pairs. The data are shown separately as well as lumped for the two years. The lumping is justified because inspection of Table 6 indicates that the patterns for the two years are similar. The lumped data will therefore be considered first, and the minor differences between the two years will be discussed later.

Text-figure 10a demonstrates the regularity with which successful courtships were based on participation in all seven phases. Of the 78 instances which led to copulation, 100% included participation in phases 1, 5, 6 and 7, 95% in phase 2, 87% in phase 3 and 82% in phase 4. Moreover, as shown by Table 7, hairpencilling occurred in either or both phases 2 and 3 in 100% of the courtships which were successful. *Thus copulation under natural conditions appears to be impossible in the complete absence of hairpencilling.* A comparison of text-figures 10a and b shows that several phases tend to be omitted in unsuccessful courtships. Compared to

95% of the successful males which hairpencilled in the air (phase 2), only 48% of the unsuccessful ones did. The difference is even greater in ground hairpencilling (phase 3), being 87% and 29% respectively. Similar drops occurred in phases 4 and 5. However, it is important to note that the proportion of unsuccessful courtships which entered the hovering and striking phase (phase 4) was greater than the proportions entering phases 2 and 3. In other words, sustained courtships occur even though phases 2 and 3 are omitted. Text-figure 10c shows the frequencies in both successful and unsuccessful courtships; this is the quantitative picture of the behavior of a population with respect to participation in the seven phases of courtship.

Several lines of evidence to be developed in the course of this analysis all point towards the conclusion that the 1961 females were both more attractive and receptive than those in 1960. However, it is here difficult to separate cause and effect. The females in some cases were more attractive because they were more receptive, but this was not always so. The differences in the unsuccessful courtships for the two years are suggestive in this respect. In 1961 a higher proportion of courtships continued through phases 2-5, while in 1960 there was consistently less participation in these phases. The only significant difference between the two years in the successful courtship category is in phase 4. This is almost certainly to be explained by the greater female receptivity and hence the by-passing of this phase to a greater degree in 1961.

### 2. Duration of the Courtship and Its Phases

The duration of each phase in an individual courtship was determined by taking the sum of the times spent in its repetitions in that courtship, and then tabulated as shown in Table 5. There it can be seen that the individual courtships were grouped into four categories: successful single first aerial component courtships, successful multiple first aerial component courtships, unsuc-

PARTICIPATION OF MALES IN SEVEN PHASES OF COURTSHIP (1960+61)

a) Successful males
(N = 78)

b) Unsuccessful males
(N = 188)

c) All males
(N = 266)
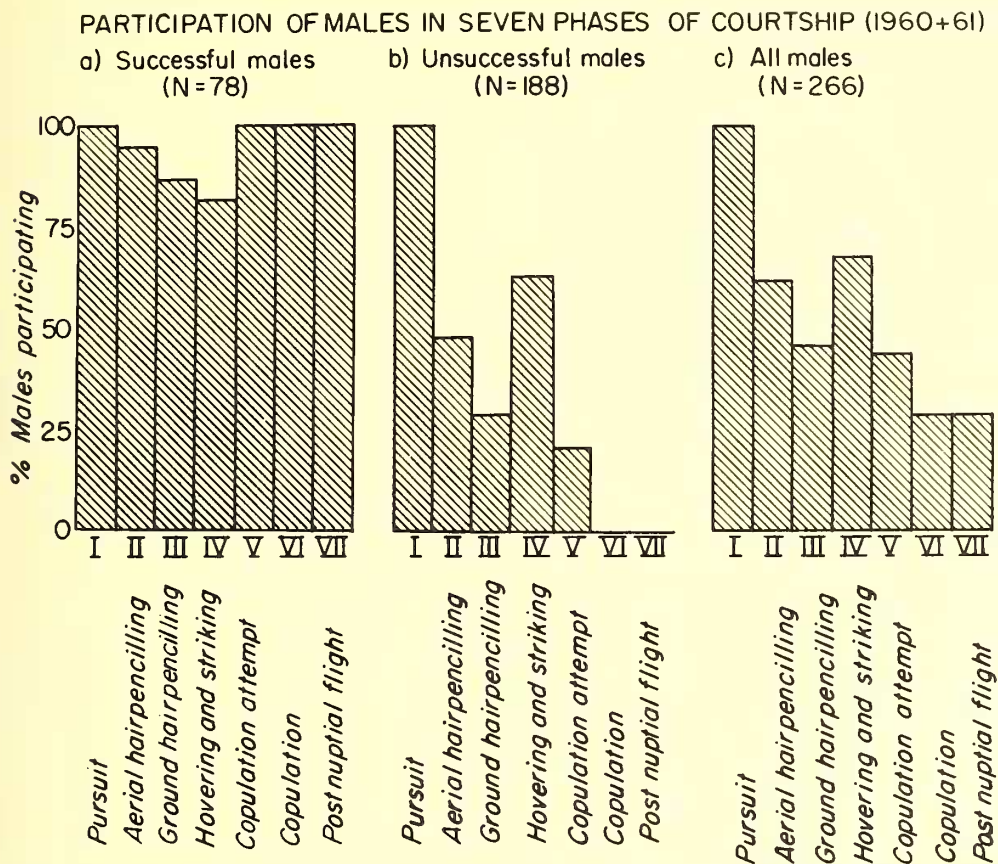


TEXT-FIG. 10 a-c. *Percentage of males participating in the seven phases of courtship.* (a) 78 successful courtships; (b) 188 unsuccessful courtships; (c) all 266 courtships representing the quantitative response pattern of a large population. Data are in Table 6.

cessful single first aerial component courtships, and unsuccessful multiple first aerial component courtships. The individual values for each phase within these categories were then added together and their means calculated. These are summarized for both years in Tables 8a-f. In addition to showing the mean duration and range of each phase of courtship, Tables 8a-f also show the time spent in the particular phase as a percentage of the total courtship time. These percentages were used to construct figures 11a-d, and the total area under each of the curves represents 100% of the courtship time. The mean durations of the entire courtship time within each of the four categories were similarly calculated and are shown in Table 8g.

Duration measurements for phases 1-4 were easily made because each of these categories was a discreet entity and did not overlap with any other. This was also true for attempts to copulate (phase 5) which were unsuccessful. However, for those which were successful, it was necessary

to choose a criterion to indicate the end of the attempt and the beginning of copulation. This was the first wing-snap of the male, which invariably occurred in successful, but never in unsuccessful, courtships. The experimental criterion taken to set the limits of the duration of copulation (phase 6) was the time between the male's first wing-snap until he flew off with the female in the post-nuptial flight (phase 7). As discussed above, this is not a measure of the total duration of copulation, which in fact continues throughout phase 8 (see Table 1). Nevertheless, the fly-off marks the end of courtship *per se* and is therefore biologically important. Moreover, since the mated pairs were captured as soon as they flew off, it was not possible to measure the time span of the post-nuptial flight (phase 7).

The mean duration for all 266 courtships was 40.4 seconds and ranged from 1 second in a courtship which consisted only of a short aerial pursuit to 317 seconds in a long successful one (Table 8g). (In another series of experiments

TABLE 5. DURATION AND NUMBER OF REPETITIONS OF PHASES OF 79 SUCCESSFUL AND
UNSUCCESSFUL COURTSHIPS OF 41 FEMALES IN 1961

All females 2-day-old virgins, experimentally courted ≤ 3 times.
Letters a-c designate 1st to 3rd time courted.

| Courtship No. | ♀ No. | Cause of Termination (See Table 2) | Duration (Seconds) of Phase 1 | 2 | 3 | 4 | 5 | 6 | Total | No. of Repetitions of Phase 1 | 2 | 3 | 5 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Successful Courtships with Single First Aerial Component (N = 12) |||||||||||||
| 2 | 24b | | 1 | 3 | 5 | 1 | 14 | 18 | 42 | 1 | 1 | 1 | 1 |
| 3 | 27a | | 1 | 5 | 1 | 1 | 29 | 1 | 38 | 1 | 1 | 1 | 1 |
| 5 | 43a | | 7 | 3 | 2 | 0 | 8 | 9 | 29 | 1 | 1 | 1 | 1 |
| 11 | 59a | | 4 | 3 | 6 | 0 | 24 | 5 | 42 | 1 | 1 | 1 | 1 |
| 12 | 65a | | 3 | 1 | 6 | 0 | 9 | 3 | 22 | 1 | 1 | 1 | 1 |
| 15 | 67c | | 5 | 6 | 10 | 0 | 7 | 17 | 45 | 1 | 1 | 1 | 1 |
| 26 | 185c | | 1 | 1 | 1 | 1 | 11 | 1 | 16 | 1 | 1 | 1 | 1 |
| 52 | 142a | | 2 | 1 | 6 | 0 | 6 | 4 | 19 | 1 | 1 | 1 | 1 |
| 60 | 221a | | 1 | 3 | 2 | 0 | 7 | 2 | 15 | 1 | 1 | 1 | 1 |
| 68 | 294a | | 2 | 3 | 2 | 0 | 9 | 1 | 17 | 1 | 1 | 1 | 1 |
| 78 | 314a | | 3 | 2 | 3 | 0 | 15 | 0 | 23 | 1 | 1 | 1 | 1 |
| 79 | 319a | | 6 | 7 | 1 | 1 | 10 | 7 | 32 | 1 | 1 | 1 | 1 |
| Successful Courtships with Multiple First Aerial Components (N = 15) |||||||||||||
| 4 | 30a | | 4 | 5 | 16 | 0 | 18 | 18 | 61 | 1 | 3 | 3 | 2 |
| 7 | 44b | | 4 | 4 | 6 | 0 | 8 | 11 | 33 | 1 | 2 | 2 | 1 |
| 19 | 114a | | 3 | 8 | 31 | 65 | 25 | 3 | 135 | 1 | 4 | 4 | 1 |
| 20 | 116a | | 23 | 20 | 9 | 74 | 10 | 3 | 139 | 5 | 5 | 4 | 1 |
| 21 | 120a | | 2 | 7 | 10 | 1 | 33 | 29 | 82 | 1 | 2 | 3 | 2 |
| 22 | 129a | | 1 | 5 | 4 | 0 | 20 | 12 | 42 | 1 | 2 | 2 | 1 |
| 23 | 135a | | 3 | 7 | 2 | 2 | 5 | 0 | 19 | 2 | 2 | 2 | 1 |
| 27 | 187a | | 3 | 11 | 3 | 1 | 8 | 17 | 43 | 2 | 2 | 2 | 1 |
| 29 | 191b | | 2 | 3 | 13 | 2 | 21 | 7 | 48 | 1 | 2 | 2 | 1 |
| 38 | 269a | | 8 | 6 | 7 | 99 | 21 | 8 | 149 | 3 | 4 | 2 | 10 |
| 39 | 330a | | 2 | 4 | 3 | 1 | 5 | 2 | 17 | 1 | 2 | 1 | 1 |
| 55 | 143c | | 5 | 4 | 5 | 245 | 29 | 16 | 304 | 3 | 2 | 3 | 12 |
| 56 | 198a | | 2 | 19 | 1 | 1 | 10 | 1 | 34 | 1 | 2 | 1 | 2 |
| 67 | 292a | | 11 | 19 | 2 | 4 | 11 | 8 | 55 | 3 | 3 | 2 | 2 |
| 71 | 297c | | 1 | 11 | 9 | 15 | 29 | 9 | 74 | 1 | 3 | 1 | 4 |
| Unsuccessful Courtships with Single First Aerial Component (N = 36) |||||||||||||
| 6 | 44a | E | 7 | 0 | 0 | 89 | 21 | 0 | 117 | 1 | 0 | 0 | 21 |
| 8 | 51a | D | 5 | 7 | 0 | 0 | 0 | 0 | 12 | 1 | 1 | 0 | 0 |
| 9 | 51b | D | 8 | 45 | 0 | 0 | 0 | 0 | 53 | 1 | 1 | 0 | 0 |
| 13 | 67a | E | 4 | 1 | 8 | 44 | 12 | 0 | 69 | 1 | 1 | 2 | 1 |
| 14 | 67b | F | 2 | 22 | 0 | 6 | 0 | 0 | 30 | 1 | 1 | 0 | 0 |
| 16 | 113a | C | 3 | 6 | 0 | 82 | 0 | 0 | 91 | 1 | 1 | 0 | 0 |
| 18 | 113c | E | 3 | 0 | 0 | 102 | 0 | 0 | 105 | 1 | 0 | 0 | 0 |
| 24 | 185a | C | 1 | 1 | 47 | 150 | 0 | 0 | 199 | 1 | 1 | 3 | 0 |
| 28 | 191a | E | 3 | 1 | 77 | 181 | 0 | 0 | 262 | 1 | 1 | 3 | 0 |
| 30 | 245a | A | 8 | 0 | 0 | 0 | 0 | 0 | 8 | 1 | 0 | 0 | 0 |
| 31 | 245b | E | 2 | 0 | 26 | 32 | 0 | 0 | 60 | 1 | 0 | 1 | 0 |
| 34 | 248b | E | 15 | 0 | 0 | 62 | 0 | 0 | 77 | 1 | 0 | 0 | 0 |
| 35 | 261a | E | 1 | 1 | 3 | 90 | 3 | 0 | 98 | 1 | 1 | 1 | 2 |
| 37 | 261c | E | 2 | 3 | 0 | 12 | 0 | 0 | 17 | 1 | 1 | 0 | 0 |
| 40 | 331a | E | 1 | 0 | 0 | 21 | 0 | 0 | 22 | 1 | 0 | 0 | 0 |
| 41 | 331b | B | 6 | 1 | 1 | 10 | 0 | 0 | 18 | 1 | 1 | 1 | 0 |
| 42 | 331c | A | 6 | 0 | 0 | 0 | 0 | 0 | 6 | 1 | 0 | 0 | 0 |
| 43 | 19a | A | 2 | 0 | 0 | 0 | 0 | 0 | 2 | 1 | 0 | 0 | 0 |
| 44 | 19b | F | 6 | 16 | 0 | 12 | 0 | 0 | 34 | 1 | 1 | 0 | 0 |
| 47 | 20b | A | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 |
| 51 | 140c | E | 1 | 2 | 4 | 17 | 1 | 0 | 25 | 1 | 1 | 1 | 1 |
| 53 | 143a | E | 1 | 1 | 5 | 22 | 0 | 0 | 29 | 1 | 1 | 1 | 0 |
| 57 | 200a | A | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 |

*continued*

TABLE 5, *continued.*

| Courtship No. | ♀ No. | Cause of Termination (See Table 2) | Duration (Seconds) of Phase 1 | 2 | 3 | 4 | 5 | 6 | Total | No. of Repetitions of Phase 1 | 2 | 3 | 5 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Unsuccessful Courtships with Single First Aerial Component (N = 36), *continued* |||||||||||||||
| 58 | 200b | B | 4 | 3 | 2 | 13 | 0 | 0 | 22 | 1 | 1 | 1 | 0 |
| 59 | 200c | E | 3 | 9 | 0 | 21 | 0 | 0 | 33 | 1 | 1 | 0 | 0 |
| 61 | 224a | A | 2 | 0 | 0 | 0 | 0 | 0 | 2 | 1 | 0 | 0 | 0 |
| 62 | 224b | B | 3 | 0 | 0 | 3 | 0 | 0 | 6 | 1 | 0 | 0 | 0 |
| 63 | 224c | B | 2 | 0 | 0 | 10 | 0 | 0 | 12 | 1 | 0 | 0 | 0 |
| 64 | 262a | B | 3 | 0 | 0 | 2 | 0 | 0 | 5 | 1 | 0 | 0 | 0 |
| 66 | 262c | F | 5 | 0 | 0 | 6 | 0 | 0 | 11 | 1 | 0 | 0 | 0 |
| 70 | 297b | F | 2 | 3 | 2 | 10 | 0 | 0 | 17 | 1 | 1 | 1 | 0 |
| 73 | 302b | E | 4 | 1 | 0 | 33 | 0 | 0 | 38 | 1 | 1 | 0 | 0 |
| 74 | 302c | E | 1 | 5 | 4 | 40 | 11 | 0 | 61 | 1 | 1 | 1 | 1 |
| 75 | 307a | A | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 1 | 0 | 0 | 0 |
| 76 | 307b | E | 1 | 0 | 43 | 99 | 61 | 0 | 204 | 1 | 0 | 2 | 10 |
| 77 | 307c | E | 1 | 0 | 0 | 71 | 15 | 0 | 87 | 1 | 0 | 0 | 10 |
| Unsuccessful Courtships with Multiple First Aerial Components (N = 16) |||||||||||||||
| 1 | 24a | F | 9 | 29 | 29 | 9 | 103 | 0 | 179 | 4 | 9 | 7 | 4 |
| 10 | 51c | F | 21 | 13 | 10 | 1 | 17 | 0 | 62 | 3 | 2 | 1 | 1 |
| 17 | 113b | E | 1 | 3 | 21 | 180 | 1 | 0 | 206 | 1 | 2 | 2 | 1 |
| 25 | 185b | E | 4 | 1 | 16 | 10 | 27 | 0 | 58 | 2 | 1 | 2 | 1 |
| 32 | 245c | E | 4 | 0 | 16 | 34 | 0 | 0 | 54 | 2 | 0 | 1 | 0 |
| 33 | 248a | E | 6 | 0 | 0 | 24 | 0 | 0 | 30 | 2 | 0 | 0 | 0 |
| 36 | 261b | E | 6 | 4 | 20 | 187 | 22 | 0 | 239 | 4 | 4 | 3 | 15 |
| 45 | 19c | E | 1 | 25 | 0 | 24 | 0 | 0 | 50 | 1 | 2 | 0 | 0 |
| 46 | 20a | E | 5 | 14 | 37 | 86 | 0 | 0 | 142 | 1 | 6 | 6 | 0 |
| 48 | 20c | B | 8 | 0 | 0 | 8 | 0 | 0 | 16 | 2 | 0 | 0 | 0 |
| 49 | 140a | B | 4 | 9 | 16 | 83 | 0 | 0 | 112 | 4 | 6 | 5 | 0 |
| 50 | 140b | E | 5 | 5 | 4 | 42 | 0 | 0 | 56 | 3 | 2 | 2 | 0 |
| 54 | 143b | C | 1 | 2 | 5 | 70 | 0 | 0 | 78 | 1 | 2 | 1 | 0 |
| 65 | 262b | E | 10 | 0 | 14 | 71 | 4 | 0 | 99 | 2 | 0 | 2 | 1 |
| 69 | 297a | F | 7 | 19 | 2 | 1 | 0 | 0 | 29 | 2 | 1 | 2 | 0 |
| 72 | 302a | F | 4 | 5 | 22 | 17 | 0 | 0 | 48 | 2 | 3 | 3 | 0 |

the longest of all courtships observed by us lasted for 410 seconds, *i.e.,* nearly 7 minutes!). A comparison of the means for successful courtships in 1960 and 1961 shows that both single and multiple aerial component courtships lasted for comparable amounts of time in the two years. However, the unsuccessful ones were dissimilar in the two years: in 1961 single and multiple aerial

TABLE 6. PARTICIPATION OF MALES IN SEVEN PHASES OF COURTSHIP

| Phase | | Successful Males 1960 | 1961 | Total | Unsuccessful Males 1960 | 1961 | Total | All Males 1960 | 1961 | Total |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | No. | 51 | 27 | 78 | 136 | 52 | 188 | 187 | 79 | 266 |
| | Freq. | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| 2 | No. | 47 | 27 | 74 | 60 | 30 | 90 | 107 | 57 | 164 |
| | Freq. | .92 | 1.00 | .95 | .44 | .58 | .48 | .57 | .72 | .62 |
| 3 | No. | 41 | 27 | 68 | 30 | 25 | 55 | 71 | 52 | 123 |
| | Freq. | .80 | 1.00 | .87 | .22 | .48 | .29 | .38 | .66 | .46 |
| 4 | No. | 48 | 16 | 64 | 75 | 43 | 118 | 123 | 59 | 182 |
| | Freq. | .94 | .59 | .82 | .55 | .83 | .63 | .66 | .75 | .68 |
| 5 | No. | 51 | 27 | 78 | 25 | 13 | 38 | 76 | 40 | 116 |
| | Freq. | 1.00 | 1.00 | 1.00 | .18 | .25 | .20 | .41 | .51 | .44 |
| 6 & 7 | No. | 51 | 27 | 78 | 0 | 0 | 0 | 51 | 27 | 78 |
| | Freq. | 1.00 | 1.00 | 1.00 | .00 | .00 | .00 | .27 | .34 | .29 |
| Total | | 51 | 27 | 78 | 136 | 52 | 188 | 187 | 79 | 266 |

TABLE 7. RELATION OF HAIRPENCILLING TO THE SUCCESS OF COURTSHIP

| Outcome of Courtship | Courtship with: | | | | | | Totals | | |
|---|---|---|---|---|---|---|---|---|---|
| | Presence of Hairpencilling | | | Absence of Hairpencilling | | | | | |
| | 1960 | 1961 | Total | 1960 | 1961 | Total | 1960 | 1961 | Total |
| Successful | 51 | 27 | 78 | 0 | 0 | 0 | 51 | 27 | 78 |
| Unsuccessful | 73 | 34 | 107 | 63 | 18 | 81 | 136 | 52 | 188 |
| Totals | 124 | 61 | 185 | 63 | 18 | 81 | 187 | 79 | 266 |
| % Successful | 41% | 44% | 42% | 0% | 0% | 0% | 27% | 34% | 29% |

Significance: by inspection, there is no significant difference between the 1960 and 1961 data, which are therefore lumped in a 2 × 2 contingency table. (Chi square = 48.5, d.f. = 1, P < .001).

component courtships both lasted for a much longer time than in 1960. This is another line of evidence that the females in 1961 were more attractive.

The average duration of all the successful courtships was 55.2 seconds (Table 8g). The shortest lasted for only 14 seconds, while the longest carried on for 317 seconds. A vast difference exists in the duration of these, depending on whether they are single or multiple aerial component courtships, the latter lasting for an average of 84.3 seconds, or nearly three times as long as the single aerial ones (30.2 seconds). Moreover, the range of single aerial courtships is much less, being 14-69 seconds compared to 17-317 seconds. The time-frequency distribution

TABLE 8a. DURATION OF AERIAL PURSUIT (PHASE 1)

| Category of Courtship | % Duration of Total Courtship | | | Time in Seconds | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Mean | | | Range | | |
| | 1960 | 1961 | Total | 1960 | 1961 | Total | 1960 | 1961 | Total |
| Successful Courtships | | | | | | | | | |
| Single Aerial | 6.4 | 10.6 | 7.5 | 2.0 | 3.0 | 2.3 | 1-5 | 1-7 | 1-7 |
| Multiple Aerial | 7.7 | 6.0 | 7.0 | 6.6 | 4.9 | 5.9 | 2-49 | 1-23 | 1-49 |
| Total Successful | 7.3 | 7.0 | 7.2 | 3.9 | 4.1 | 4.0 | 1-49 | 1-23 | 1-49 |
| Unsuccessful Courtships | | | | | | | | | |
| Single Aerial | 18.0 | 6.8 | 12.6 | 3.0 | 3.5 | 3.1 | 1-26 | 1-15 | 1-26 |
| Multiple Aerial | 7.1 | 6.6 | 6.8 | 4.7 | 6.0 | 5.4 | 2-8 | 1-21 | 1-21 |
| Total Unsuccessful | 14.0 | 6.7 | 10.3 | 3.2 | 4.3 | 3.5 | 1-26 | 1-21 | 1-26 |
| Total Courtships | 10.8 | 6.8 | 9.0 | 3.4 | 4.2 | 3.6 | 1-49 | 1-23 | 1-49 |

TABLE 8b. DURATION OF AERIAL HAIRPENCILLING (PHASE 2)

| Category of Courtship | % Duration of Total Courtship | | | Time in Seconds | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Mean | | | Range | | |
| | 1960 | 1961 | Total | 1960 | 1961 | Total | 1960 | 1961 | Total |
| Successful Courtships | | | | | | | | | |
| Single Aerial | 15.7 | 11.2 | 14.5 | 4.9 | 3.2 | 4.4 | 0-35 | 1-7 | 0-35 |
| Multiple Aerial | 18.8 | 10.8 | 15.6 | 16.1 | 8.9 | 13.1 | 0-167 | 3-20 | 0-167 |
| Total Successful | 17.8 | 10.9 | 15.2 | 9.5 | 6.3 | 8.4 | 0-167 | 1-20 | 0-167 |
| Unsuccessful Courtships | | | | | | | | | |
| Single Aerial | 13.2 | 7.0 | 10.2 | 2.2 | 3.6 | 2.5 | 0-13 | 0-45 | 0-45 |
| Multiple Aerial | 9.5 | 8.8 | 9.1 | 6.4 | 8.1 | 7.2 | 0-32 | 0-29 | 0-32 |
| Total Unsuccessful | 11.9 | 7.8 | 9.8 | 2.7 | 4.9 | 3.4 | 0-32 | 0-45 | 0-45 |
| Total Courtships | 14.6 | 8.8 | 12.0 | 4.6 | 5.4 | 4.8 | 0-167 | 0-45 | 0-167 |

TABLE 8c. DURATION OF GROUND HAIRPENCILLING (PHASE 3)

| Category of Courtship | % Duration of Total Courtship | | | Time in Seconds | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | | | | Mean | | | Range | | |
| | 1960 | 1961 | Total | 1960 | 1961 | Total | 1960 | 1961 | Total |
| Successful Courtships | | | | | | | | | |
| Single Aerial | 5.5 | 13.2 | 7.6 | 1.7 | 3.8 | 2.3 | 0-13 | 1-10 | 0-13 |
| Multiple Aerial | 7.1 | 9.8 | 8.2 | 6.1 | 8.1 | 6.9 | 0-26 | 1-31 | 0-31 |
| Total Successful | 6.5 | 10.5 | 8.0 | 3.5 | 6.2 | 4.4 | 0-26 | 1-31 | 0-31 |
| Unsuccessful Courtships | | | | | | | | | |
| Single Aerial | 5.5 | 12.1 | 8.7 | 0.9 | 6.2 | 2.1 | 0-16 | 0-77 | 0-77 |
| Multiple Aerial | 12.7 | 14.5 | 13.7 | 8.6 | 13.3 | 10.9 | 0-29 | 0-37 | 0-37 |
| Total Unsuccessful | 8.1 | 13.2 | 10.7 | 1.9 | 8.3 | 3.7 | 0-29 | 0-77 | 0-77 |
| Total Courtships | 7.4 | 12.3 | 9.6 | 2.3 | 7.6 | 3.9 | 0-29 | 0-77 | 0-77 |

TABLE 8d. DURATION OF HOVERING AND STRIKING (PHASE 4)

| Category of Courtship | % Duration of Total Courtship | | | Time in Seconds | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | | | | Mean | | | Range | | |
| | 1960 | 1961 | Total | 1960 | 1961 | Total | 1960 | 1961 | Total |
| Successful Courtships | | | | | | | | | |
| Single Aerial | 4.4 | 1.2 | 3.5 | 1.4 | 0.3 | 1.1 | 0-4 | 0-1 | 0-4 |
| Multiple Aerial | 30.0 | 41.3 | 34.6 | 25.7 | 34.0 | 29.2 | 2-208 | 0-245 | 0-245 |
| Total Successful | 21.3 | 32.6 | 25.5 | 11.4 | 19.0 | 14.0 | 0-208 | 0-245 | 0-245 |
| Unsuccessful Courtships | | | | | | | | | |
| Single Aerial | 55.1 | 67.4 | 61.0 | 9.2 | 34.4 | 15.1 | 0-115 | 0-181 | 0-181 |
| Multiple Aerial | 66.1 | 58.1 | 61.6 | 44.6 | 52.9 | 48.7 | 2-97 | 1-187 | 1-187 |
| Total Unsuccessful | 59.2 | 63.3 | 61.3 | 13.6 | 40.1 | 21.0 | 0-115 | 0-187 | 0-187 |
| Total Courtships | 41.5 | 53.4 | 46.9 | 13.0 | 32.9 | 18.9 | 0-208 | 0-245 | 0-245 |

TABLE 8e. DURATION OF COPULATION ATTEMPT (PHASE 5)

| Category of Courtship | % Duration of Total Courtship | | | Time in Seconds | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | | | | Mean | | | Range | | |
| | 1960 | 1961 | Total | 1960 | 1961 | Total | 1960 | 1961 | Total |
| Successful Courtships | | | | | | | | | |
| Single Aerial | 24.9 | 43.8 | 29.9 | 7.7 | 12.4 | 9.0 | 3-18 | 6-29 | 3-29 |
| Multiple Aerial | 22.3 | 20.5 | 21.6 | 19.1 | 16.9 | 18.2 | 3-155 | 5-33 | 3-155 |
| Total Successful | 23.2 | 25.5 | 24.1 | 12.4 | 14.9 | 13.3 | 3-155 | 5-33 | 3-155 |
| Unsuccessful Courtships | | | | | | | | | |
| Single Aerial | 8.2 | 6.7 | 7.5 | 1.4 | 3.4 | 1.9 | 0-43 | 0-61 | 0-61 |
| Multiple Aerial | 4.6 | 11.9 | 8.7 | 3.1 | 10.9 | 6.9 | 0-25 | 0-103 | 0-103 |
| Total Unsuccessful | 6.9 | 9.0 | 8.0 | 1.6 | 5.7 | 2.7 | 0-43 | 0-103 | 0-103 |
| Total Courtships | 14.5 | 14.4 | 14.4 | 4.5 | 8.9 | 5.8 | 0-155 | 0-103 | 0-155 |

for successful single aerial component courtships is nearly normal (*i.e.*, Gaussian) whereas that for successful multiple aerial ones is heavily skewed to the right (Tables 5, 8g).

Moreover, this normal distribution is not characteristic of single aerial courtships which are unsuccessful. These, as well as the unsuccessful multiple courtships, are skewed in a fashion simi-

TABLE 8f. DURATION OF COPULATION-POST-NUPTIAL FLIGHT (PHASE 6)

| Category of Courtship | % Duration of Total Courtship | | | Time in Seconds | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Mean | | | Range | | |
| | 1960 | 1961 | Total | 1960 | 1961 | Total | 1960 | 1961 | Total |
| Successful Courtships | | | | | | | | | |
| Single Aerial | 43.2 | 20.0 | 37.0 | 13.4 | 5.7 | 11.2 | 1-31 | 0-18 | 0-31 |
| Multiple Aerial | 14.0 | 11.7 | 13.1 | 12.0 | 9.6 | 11.0 | 1-41 | 0-29 | 0-41 |
| Total Successful | 23.9 | 13.5 | 20.1 | 12.8 | 7.9 | 11.1 | 1-41 | 0-29 | 0-41 |
| Unsuccessful Courtships | | | | | | | | | |
| Single Aerial | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0 | 0 | 0 |
| Multiple Aerial | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0 | 0 | 0 |
| Total Unsuccessful | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0 | 0 | 0 |
| Total Courtships | 11.1 | 4.4 | 8.1 | 3.5 | 2.7 | 3.3 | 1-41 | 0-29 | 0-41 |

TABLE 8g. TOTAL DURATION OF COURTSHIP (PHASES 1-6)

| Category of Courtship | Number of Males | | | Time in Seconds | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Mean | | | Range | | |
| | 1960 | 1961 | Total | 1960 | 1961 | Total | 1960 | 1961 | Total |
| Successful Courtships | | | | | | | | | |
| Single Aerial | 30 | 12 | 42 | 31.0 | 28.3 | 30.2 | 14-69 | 15-45 | 14-69 |
| Multiple Aerial | 21 | 15 | 36 | 85.7 | 82.3 | 84.3 | 23-317 | 17-304 | 17-317 |
| Total Successful | 51 | 27 | 78 | 53.5 | 58.3 | 55.2 | 14-317 | 15-304 | 14-317 |
| Unsuccessful Courtships | | | | | | | | | |
| Single Aerial | 119 | 36 | 155 | 16.7 | 51.1 | 24.7 | 1-125 | 1-262 | 1-262 |
| Multiple Aerial | 17 | 16 | 33 | 67.5 | 91.1 | 79.0 | 5-143 | 16-239 | 5-239 |
| Total Unsuccessful | 136 | 52 | 188 | 23.0 | 63.4 | 34.2 | 1-143 | 1-262 | 1-262 |
| Total Courtships | 187 | 79 | 266 | 31.4 | 61.7 | 40.4 | 1-317 | 1-304 | 1-317 |

lar to the successful multiple ones, the mean of the single being 24.7 and ranging from 1-262 seconds, and the mean of the multiple being 79.0 and ranging from 5-239 seconds. There is, in other words, a qualitative difference between successful single aerial component courtships and the rest; they tend very rapidly to proceed to completion, whereas in the others the sequence is broken, individual phases are repeated and a disproportionate amount of time is spent in phase 4.

Considering now the time spent in the individual phases of courtship, we see that phases 1 and 2 are roughly comparable for all four categories in 1960 and 1961 (Tables 8a and b). However, phases 3-5 (Tables 8c-e) are generally longer in 1961 than in 1960, while the reverse is true for phase 6 (Table 8f). Thus in 1961, once a courtship began, more time tended to be spent in ground hairpencilling, hovering and striking, and attempting to copulate than in 1960. This again suggests that the 1961 females were

more attractive than those in 1960. Figures 11a-d, based on Tables 8a-f, summarize the data for the two years and illustrate that the first three phases all take similar percentages of time irrespective of whether the courtships are successful, unsuccessful, single, or multiple aerial component; the range is also small, from 7.0 to 15.6% (2.1 to 13.1 seconds). However, the hovering and striking (phase 4) is extremely variable, from 3.5 to 61.6% (1.1 to 48.7 seconds), and its analysis in relation to the four kinds of courtship is of the utmost importance in illuminating its functional significance. Text-figure 11a is a graph of the percentage of time spent in each phase in all 266 courtships. This shows that as a population the butterflies spend over three-fold the amount of time in phase 4 than in any other (46.9% in phase 4 compared to 14.4% in the next largest, phase 5). Text-figure 11b separates the unsuccessful from the successful courtships and shows that the unsuccessful males spend more time (61.3%) hovering and striking. Nev-
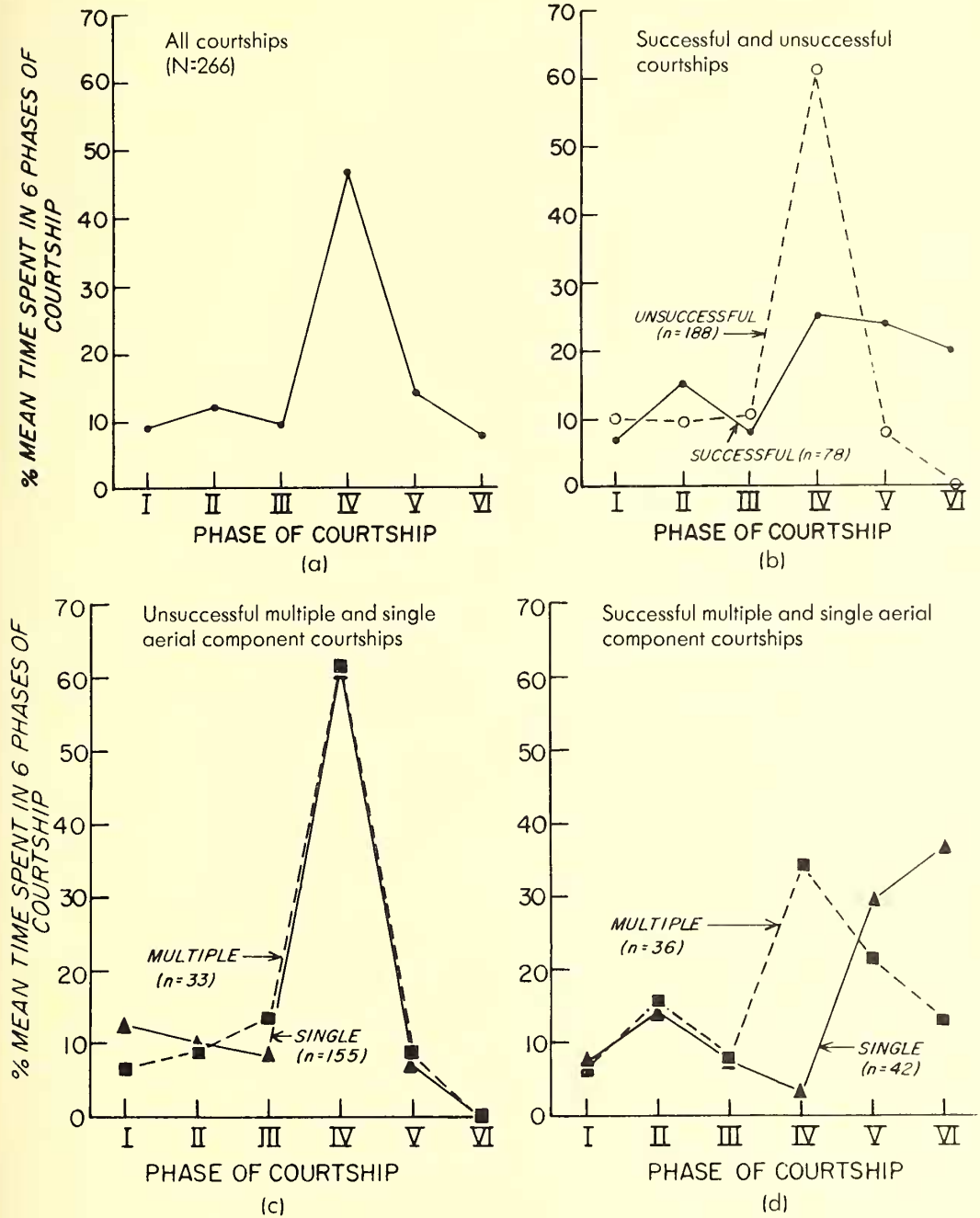
TEXT-FIG. 11 a-d. *Analysis of the average time spent in the first six phases of courtship*. The duration of each phase is expressed as a percentage of the total courtship time which equals the entire area beneath each curve. **(a)** All courtships; **(b)** comparison of successful with unsuccessful courtships; **(c)** comparison of multiple with single aerial component courtships which are unsuccessful; **(d)** comparison of multiple with single aerial component courtships which are successful. See text for interpretation. Data are in Tables 8a-8f.

ertheless, the successful ones do spend a substantial portion of time (25.5%) in this phase. Text-figure 11c breaks down the unsuccessful courtships into those with single or multiple aerial components and shows that the percentage of time spent hovering and striking in both is vir-

tually the same. However, this is not true of successful courtships as shown in text-figure 11d. Hovering and striking is negligible in single aerial component courtships (3.5% of the time) whereas in multiple aerial component ones it occupies the major portion of the time (34.6%). In fact, phase 4 in the successful single aerial courtships consisted mainly of transitional hovering lasting only one second and occurring between phases 2 and 3 or 5. Moreover, in these instances the striking component was absent.

Since phase 4 is such a negligible feature in these successful single aerial courtships which are obviously the most efficient from the point of view of time and energy expended, why then is hovering and striking so prominent a feature in all other courtships? The answer to this is found partly in Table 4 which indicates that multiple aerial courtships are more often successful than single aerial ones (P <.001). It was clear in observing the behavior of the butterflies that the hovering and striking occurred either when the males omitted phases 2 and 3 or when the females did not quickly fold their wings and become receptive. Although at first unsuccessful, the chances were high that mating could occur if the male could sustain the courtship long enough to reinduce the aerial component as indicated by Table 4. In other words, hovering and striking, as well as the dorsal attempts at copulation, are functionally important because they either directly stimulate the female to take flight again, or they keep the male close enough to the female so that when she does fly off he can reinitiate the courtship.

The average proportion of time spent in attempting to copulate (phase 5, Table 8e) in all courtships is 14.4% (5.8 seconds), in successful courtships 24.1% (13.3 seconds) and in unsuccessful ones 8% (2.7 seconds). Little difference exists between single and multiple aerial component courtships within the successful and unsuccessful categories. Finally, the time spent in copulation (phase 6, Table 8f) was 20.1% (11.1 seconds) for all successful males. It tended to represent a much higher proportion of time of the single aerial courtships (37%) than for the multiple aerial ones (13.1%), but, as would be expected, the actual time spent in this phase was nearly equal for both (11.2 and 11.0 seconds, respectively).

### 3. *Sequence and Repetition of Phases in the Courtship*

In Table 3 the sequence and repetition pattern for the phases of successful courtships in 1961 are summarized and it can be seen once again that there is a qualitative difference between sin-

gle aerial and multiple aerial component courtships. Single aerial ones are highly regular and with the exception of phase 4 (hovering and striking), proceed in succession through the seven phases. In 8 of the 12 courtships, phase 4 was omitted altogether; and in the remaining four it preceded phase 3; and as noted above, in these it consisted only of a period of approximately one second during the transition from aerial to ground hairpencilling as the female alighted. Moreover, each phase occurred only once in all of these courtships, and this was also true of all but one of the 30 single aerial courtships in 1960, in which the sequence was 1, 2, 4, 3, 4, 5, 6, 7.

In contrast, the multiple aerial component courtships involved variable repetition of all the phases, although even in these the normal sequence tends to be preserved. As seen in the table, this is particularly true both near the beginning and the end of the courtship. Furthermore, in the terminal portion, phase 4 is often omitted as it is in successful single aerial courtships. The longest courtship in 1961 (no. 143c), which lasted for 304 seconds, is illuminating in this respect. This proceeded through the first 5 phases but then became very prolonged as the male alternated between hovering and striking the female and attempting to copulate with her. Finally, after twice inducing her to fly off, the courtship progressed rapidly through the normal sequence to copulation.

It is thus evident that the sequence and repetition of the phases leading to copulation is highly stereotyped in the courtship of the Queen butterfly, and it is clear that multiple aerial component courtships are an elaboration of the basic single aerial courtship in which phase 4 is particularly extended and repeated.

### 4. *Unsuccessful Courtships*

In Table 2 the data for unsuccessful courtships in 1960 and 1961 are summarized, and it can be seen that termination by the male or the female occurred to about the same extent. Aerial dismissal by the male during phase 1 or 2 was responsible for ending 27% of the courtships. Of nearly equal frequency were desertion by the male and rejection by the female during the ground component. Of somewhat less importance, but nevertheless significant, was evasion by the female either directly in the air or by flying through foliage. These accounted for, respectively, 6% and 10% of the unsuccessful courtships. Finally, homocourtship brought the sequence to an end in 8% of the cases.

### 5. *Lateral and Dorsal Copulation Attempts*

The only successful copulation attempts (phase

5) are those that take place laterally (Table 9a). For both years, 250 attempts to copulate were made of which 139 were lateral and 111 dorsal. Fifty-six per cent of the lateral attempts were successful, whereas none of the dorsal ones was. The fact that the number of attempts from the two positions did not depart significantly from equality indicates the magnitude of importance of the dorsal ones even though they never lead to copulation *per se*. As already discussed, their functional significance, together with hovering and striking, is to reinduce the first aerial component of the courtship and thereby greatly increase the probability of achieving copulation.

Table 9b is an analysis of right and left lateral copulation attempts and shows clearly that both occur with a similar frequency and both are about equally successful. There is thus no tendency for asymmetry in mating position in the Queen butterfly.

## VII. Discussion

### (A). *Courtship of the Queen Compared with Other Danaines*

Observations of other danaine butterflies suggest that their courtships are broadly similar to that of the Queen, but sufficient data are not available for quantitative comparisons. An incomplete courtship of *Limnas chrysippus* was noted by Marshall (1902) in which the male was hovering above and intermittently dropping dorsally onto a female as she clung to vegetation and fluttered her wings. This appears comparable to phase 4 of the Queen and lasted for about 5 minutes before ending in homocourtship. What appears similar to phase 3 was subsequently observed by Carpenter (*in* Carpenter & Poulton, 1927). The male was seen hovering about four inches above and in front of the female, rapidly protruding and withdrawing its hairpencils. How-

TABLE 9. RELATION OF MALE POSITION DURING COPULATION ATTEMPT
(PHASE 5) TO SUCCESS OF COURTSHIP

### a. Lateral vs. Dorsal Copulation Attempts

| Outcome of Courtship | Position of Male with Respect to Female | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Lateral | | | Dorsal | | | Totals | | |
| | 1960 | 1961 | Total | 1960 | 1961 | Total | 1960 | 1961 | Total |
| Successful | 51 | 27 | 78 | 0 | 0 | 0 | 51 | 27 | 78 |
| Unsuccessful | 33 | 28 | 61 | 43 | 68 | 111 | 76 | 96 | 172 |
| Totals | 84 | 55 | 139 | 43 | 68 | 111 | 127 | 123 | 250 |
| % Successful | 61% | 49% | 56% | 0% | 0% | 0% | 40% | 22% | 31% |

*Significance:* (1) by inspection there is no significant difference between the 1960 and 1961 data; in both years copulation was achieved only from the lateral position ($P < .001$).

(2) in 1960, significantly more lateral than dorsal attempts to copulate were made (Chi square $= 13.24$, d.f. $= 1$, $P < .001$). In 1961, the apparent reversal of this tendency was not significant ($\chi^2 = 1.38$; d.f. $= 1$; $.30 > P > .20$). When the data for the two years are lumped, there is no significant difference in lateral and dorsal attempts to copulate ($\chi^2 = 3.14$; d.f. $= 1$; $.10 > P > .05$).

### b. Right vs. Left Lateral Copulation Attempts

| Outcome of Courtship | Position of Male with Respect to Female | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Right Lateral | | | Left Lateral | | | Totals | | |
| | 1960 | 1961 | Total | 1960 | 1961 | Total | 1960 | 1961 | Total |
| Successful | 30 | 12 | 42 | 19 | 15 | 34 | 49 | 27 | 76 |
| Unsuccessful | 11 | 19 | 30 | 12 | 9 | 21 | 23 | 28 | 51 |
| Totals | 41 | 31 | 72 | 31 | 24 | 55 | 72* | 55 | 127 |
| % Successful | 73% | 39% | 58% | 61% | 63% | 62% | 68% | 49% | 60% |

*Discrepancy from 84 in Table 9a due to omission in original tape records in 12 instances.

*Significance:* (1) the apparent greater success from the right in the 1960 data is not significant (Chi square $= 1.16$; d.f. $= 1$; $.30 > P > .20$). The apparent greater success from the left in the 1961 data is also not significant (Chi square $= 2.19$; d.f. $= 1$; $.20 > P > .10$). When the 1960 and 1961 data are lumped, there is no significant difference (Chi square $= .162$; d.f. $= 1$; $.70 > P > .50$).

(2) Attempts to copulate from the left or right do not depart significantly from a .5 right: .5 left expectation for both years (1960: Chi square $= 1.38$; d.f. $= 1$; $.30 > P > .20$; 1961: Chi square $= .90$; d.f. $= 1$; $.50 > P > .30$).

ever, the female was unreceptive and apparently terminated the courtship by foliage evasion. An experimental investigation on this species was carried out by Stride (1958a). Having isolated eight males in an insectary for six days, he then released six females into the cage, and the males immediately commenced courting them. According to Stride, the female normally flies in an unhurried manner, but this is replaced in courtship by a rapid, rather jerky flight consisting of a series of short dashes. This appears to be different from the Queen, but from then on their courtship seems to be similar. "The male flew above the female, and each time opportunity offered he dipped down to strike the front part of the female with his anal brushes. In a short time the female settled on a leaf or the side of the cage and the male settled beside her, facing the same direction. Copulation was effected by a flexion of the male abdomen in a forward direction. Any further movement on the part of the paired butterflies was effected by the male . . ." (p. 229). In the cage several males simultaneously hairpencilled a female but this group activity usually ended in homocourtship.

Urquhart (1958, 1960) has described various aspects of the courtship of the Monarch butterfly from which it may be inferred that the aerial pursuit phase is similar to the Queen's but that the aerial hairpencilling phase is quite different. As the male pursues the female, she flies from him in a spiral flight. He then overtakes and apparently hairpencils her in small circles, the pair thus rising in a vertical spiral. Neither ground hairpencilling nor hovering and striking was noted. However, it may be that what Urquhart described as a walking phase is comparable to the hovering and striking phase of the Queen since both occur when the females are unreceptive in the ground component. In this the male Monarch struts in front of the female and opens and closes his wings while she remains stationary with her wings folded dorsally or only slightly opened. Simultaneously, she was often seen extending her proboscis as if to feed, which Urquhart speculates is in response to a flowerlike scent emanating from the male's wing pockets. We did not observe this in the Queen. Copulation followed lateral attempts as in the Queen. Three aspects of elusive behavior by the female also appeared similar to the Queen: aerial evasion during the pursuit, wing thrusting and dorsal twisting of the abdomen during the copulation attempt.

Two incomplete courtships in *Amauris psyttalea* Plötz were noted by Carpenter (*in* Carpenter & Poulton, 1914, 1929) in Africa. A male was seen (1914) pursuing a female which settled with her wings open on a dead flower stalk. The male then hovered about four inches over her head, rising and falling a little, but on the whole at about the same level. During this his abdomen hung down and at intervals of a few seconds he rapidly extruded and withdrew the hairpencils. The courtship went on for about a minute before the female flew away, pursued by the male. During the hairpencilling the female sat quietly except for an occasional slight movement of her wings, which she apparently kept open the whole time. Presumably, the very sudden protrusion of the hairpencils and equally rapid withdrawal causes the dust produced in enormous quantities by this species to sprinkle forth over the anterior of the female. The later observation (1929) was less complete than this and the only difference was that the female was pursued by several males prior to her settling. Apparently the only other recorded courtship of danaines is that of *Tirumala limnace,* observed by Punnet (*in* Poulton & Punnet, 1927) and again it is an incomplete observation of a pair in the ground component in which the female seemed to be unreceptive. The use of the hairpencils was not noted.

### (B). *Courtship of Euploeines and Lycoreines*

Our knowledge of euploeine courtship is even more limited, only *Euploea core asela* having been observed. According to Latter & Eltringham (1935), the female flies to the male and the pair then fly about each other through the air, settle on herbage and mate. Sevastopulo (*in* Sevastopulo & Carpenter, 1944) noted a male hovering about two feet above a female which was sitting on a leaf with her wings closed. During this, the male protruded and withdrew its hairpencils, and every few seconds flew lower and buffeted the female. This continued until the female flew away closely followed by the male. Thus there is evidence that the Euploeini differ from the Danaini in that the female is attracted to the male; more will be said about this below (section D). Otherwise, the fragmentary observations suggest that the behavior of these two tribes is similar.

Observations of lycoreine courtship are completely lacking.

### (C). *Stimuli Involved in the Courtship*

In the absence of experimental studies in which artificial dummies or machines (Magnus, 1958) are employed, it is not possible to be certain what stimuli are acting, and at what time they are effective. However, it seems valuable in the light of what is known about other butterflies, to attempt a logical analysis of what stimuli are involved in the courtship of the Queen.

Let us begin by examining the role of the visual stimuli. It seems reasonably certain that the Queen male is initially attracted to the female by seeing her in flight or while she is fluttering at rest on herbage. Urquhart has noted that Monarch males pursue numerous species of butterflies in the wild, provided they are large enough, and Magnus (1963) has argued forcefully for a relatively unspecific but definitely visual initial stimulus for butterflies in general. During the course of our observations, we released a few females of *Limenitis archippus floridensis* (Strecker) to Queen males and found that they pursued these and even hairpencilled one in the air. This species is a member of a different subfamily, the Nymphalinae, and although very similar to the Queen in appearance (it is in fact a mimic of the Queen), it must offer numerous other general and specific stimuli. The importance of size, pattern, type of movement and color remain to be determined by experiments along the lines of those carried out by Tinbergen *et al.* (1942), Magnus (1950, 1958), Petersen, Törnblom & Bodin (1952), Petersen & Tenow (1954), Crane (1955, 1957), Stride (1956, 1958a & b), Lederer (1960) and Ford (1962). However, slight changes in color-pattern seem unimportant. A series of experiments was conducted in which females were painted either to eliminate the white spotting on the forewings or to increase its area on the forewings. Courtship ended in copulation as often with these color-pattern modifications as with the painted controls (Brower, 1963).

The role visual stimuli play in the later stages of courtship is unknown, but they are probably involved whenever the male has to pursue the female through the air for more than a few inches. The fact that a female could easily evade the male at a short distance by flying through herbage supports this, as does his response to a female during his wide hovering and her intermittent fluttering in prolonged unsuccessful courtships. Moreover, termination by homocourtship suggests a conflict of objects offering similar visual stimuli. The female Queen also apparently responds visually to the male during the first aerial component of the courtship. Our interpretation is that her first reaction to the pursuing male is to avoid the visual stimulus he presents by initiating a generalized escape flight which as described above is usually vigorous and sustained. The very great modification of her behavior once she is overtaken and hairpencilled by the male in the air supports this and appears to represent a change from visual to chemical or chemo-tactic stimuli. The fact that no courtships were successful unless hairpencilling occurred, in addition to the fact that these organs are scented, argues strongly in favor of the idea that scent is the dominant stimulus to the female at this time. A bioassay technique must be developed to verify this because the possibility that the hairpencilling is exclusively a mechanical stimulus has not yet been disproved.

Whether the Queen female seeks out males by looking for them as other species do (Lederer, 1960) is unknown, as is the question of whether the male's specific color-pattern is of importance to her in the early or later stages of the courtship. There are cogent reasons for thinking that the male's color-pattern is of great importance in some butterflies (Brower, 1963).

It is difficult to ascertain what stimuli the male is responding to during the hairpencilling phase of the courtship, but it seems more likely that he is visually or tactilely and not chemically oriented to the female. Our observation of a male hairpencilling a *Limenitis* female in the air suggests this, as does Stride's (1958a) observation of a male *L. chrysippus* hairpencilling a dead *Hypolimnas misippus* Linnaeus female (Nymphalinae) which he held in his hand. The vigorous movements of the male in orienting to copulate with the female almost certainly stimulate both partners tactilely. The fact that the male's tarsi usually cling to the undersides of the female's wings at this stage could offer additional tactile stimulation and at the same time convey chemo-tactic information to the male. The palpation of the female's antennae and head by the male's antennae could similarly convey chemotactic information, or it could be simply mechanical stimulation (see Magnus, 1950). The antennae of the two sexes might also be sensing volatile substances emanating from each other.

The possible role of auditory stimuli arising from the buffeting contact of the two butterflies during the aerial and ground hairpencilling should also be investigated, as sound apparently does play a part in the courtship of some species (Müller, 1878).

### (D). *Function of the Hairpencilling*

If the interpretation presented in the last section proves to be correct, then the hairpencil perfume of the Queen male is to be regarded as a pheromone which acts as a *chemical arrestant* of the female's escape flight from the pursuing male. This follows the useful terminology set forth by Dethier, Browne & Smith (1960). As such, the perfume would be a proximity stimulus and would serve not only to arrest the female's flight, but also to inhibit her from flying away from the male once she had been induced to alight.

In the Euploeini, the function of the hairpencils may include an aspect which is completely different from the danaines. Several observations have been made, mostly on *Euploea core*, in which males have been seen patrolling certain areas *with their hairpencils fully extended in the complete absence of females* (de Niceville, 1872, *in* Clark, 1926; Champion & Poulton, 1930; Fyson & Poulton, 1930; Sevastopulo & Carpenter, 1944). Latter & Eltringham (1935) have provided evidence that this behavior attracts females from a distance. Courtship then proceeds in the normal danaine manner. More work is needed to verify that the hairpencils are attractive and if so that they act chemically and not visually, since these organs in the euploeines are extremely conspicuous when extended. If visual attraction is not involved, then the question arises, are the males producing a single scent which is both an attractant at a distance and an arrestant when applied in the normal hairpencilling, or are two chemicals produced, perhaps one by the hairpencils and one by the wing glands?

(E). *Functional Role of the Hairpencil—Wing Gland Interaction*

The physiological role that the interaction between these two organs may serve involves several possibilities, some of which can be eliminated, at least in the Queen butterfly.

It is unreasonable to accept the original hypothesis put forward by Müller (1877b) which so greatly influenced the thinking of all subsequent investigators. That is to say, it is very doubtful (1) that the wing glands *per se* produce the odoriferous substance and that the hairpencils *per se* distribute it, both because the hairpencils are complex secretory organs and as shown elsewhere (Brower & Jones, 1965) produce scent even when the wing pockets are sealed from birth.

Let us therefore consider the reverse hypothesis, namely (2) that the hairpencils *per se* secrete the scent and the wing glands *per se* disseminate it. This seems ruled out by the fact that the hairpencilling behavior is a prerequisite to successful courtship and by the form of the hairpencils when they are splayed, which on theoretical grounds alone makes them highly efficient distributive structures.

A third possibility, originally put forward by Eltringham (1935) is (3) that although only the hairpencils smell noticeably to human beings, both they and the wing glands produce substances which play completely independent and non-interacting roles in stimulating the female. This seems unlikely in view of the male's behavior in which he juxtaposes the two glands. Furthermore, in the Queen butterfly, new evidence suggests that the secretions of the two glands do interact (see hypothesis 6 below).

This leads to Urquhart's (1958, 1960) suggestion (4) that only the hairpencils produce the scent, but that both glands disseminate it. This author reasoned that the perfume must be present throughout the courtship. Since the hairpencils are withdrawn during the attempt to copulate, they could not directly stimulate the female at this time. But, if the juxtaposition of the two glands prior to courtship transfers some of the hairpencil perfume to the wing glands, then the scent would be continuously present and copulation could ensue. However, this storage hypothesis seems doubtful, first of all because the scent is undetectable or extremely faint in the wing glands, but even more importantly by the fact that these glands are also complex secretory organs[6].

If the scent of the hairpencils is distributed adhering to a dust-like material, as it almost certainly is in some species (and perhaps in all, see sections IV-C and D), then another possibility exists. It may well be (5) that the odor has a high evaporation rate which is lowered by chemically combining with a wing gland secretion. This would allow the gradual release of the scent from the dust which fell on the antennae of the female and so prolong its effect during the critical final phases of courtship when the hairpencils are withdrawn. Blum & Traynham (1960) put forward a similar hypothesis to explain the existence of two secretory components in the defensive glands of the pentatomid bug *Oebalus pugnax* (Fab.). Presumably one is the active product and the other slows its rate of evaporation and so lengthens the life of the defensive secretion in the area where it has been ejected.

Alternatively, (6) the different secretions may combine chemically[7] as a third product which

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[6]As pointed out in section IV-D, Urquhart is incorrect in his *histological interpretation* that the wing gland is a scent receptor. Furthermore, based on our experience in removing hairpencils for chemical analyses, the hairpencil "fluid" which he experimentally observed being "absorbed" by the wing gland is the yellow hemolymph of the butterfly squeezed out through the ruptured stalk of the hairpencil and is certainly *not* the hairpencil secretion.

[7]Brower & Jones (1965) have produced two lines of evidence supporting a chemical interaction of the glands in the Trinidad Queen. Wing pockets of reared males were sealed from birth, and 5 days later the hairpencil scent was of a different quality and lower intensity than in a series of control males. Wild individuals of various ages were similarly treated and 7 days later the scent of their hairpencils was found to have diminished both in strength and fragrance compared to control males.

has greater stimulative power than either alone. This hypothesis was also originally suggested by Eltringham (1929) for the males of a brassolid butterfly which has two different kinds of abdominal glands. In his words, "whether these two separate organs give rise to different scents at different times, [hypothesis 3 of the present paper] or whether their volatile products combine in the air to produce a single effect, we do not know" (p. 1). He later (1935) extended the idea of a combined product to *Euploea core asela*. Recent biochemical studies have demonstrated that certain insects do produce and store secretions in different organs and then mix them to produce a third product. For example, the miniature explosions of the Bombadier beetles of the genus *Brachinus* result from a mixture of chemicals (Schildknecht & Holoubek, 1961). Another example is seen in the cockroach, *Periplaneta americana* Linnaeus. The female of this species has paired lateral abdominal glands, one of which produces an enzyme and the other a substrate. When these are released inside the reproductive duct of the female, an organic acid is formed which tans the cuticle of the oothecum (Brunet & Kent, 1955).

Finally (7) the wing gland secretion may serve only as a preening substance for the hairpencils.

Thus it can be seen that the functional relationship of the hairpencils and wing glands in these butterflies has still to be solved. Fortunately, the analytical tools are now available to do this, and it is hoped that work in progress (Brower, Eisner & Meinwald) will shed more light on this fascinating problem.

(F.) *Speculation*

If it can be demonstrated experimentally that the male sex perfume of the Danainae functions biologically as an arrestant pheromone of the female's flight, then a whole new field of evolutionary biochemistry will be opened. Males of one species would be able to arrest their own females but might not be able to arrest those of other species. In other words, each species may have its own chemical language. Moreover, if the active arresting principles for a series of species can be chemically characterized, it may be possible to reconstruct how changes at a molecular level have resulted in the evolution of a sexual isolating mechanism. Hybridization studies could then be made to gain an understanding of the hereditary basis of the changes, and comparisons of subspecies and populations from various parts of clines would yield knowledge of how this chemical evolution occurs in nature.

It is, however, well to remember that the hair-pencilling behavior is only part of the whole courtship sequence of these butterflies. Consequently, as in the sex attractants produced by female saturniid moths (Schneider, 1962; Wilson & Bossert, 1963), the chemical specificity may not be a simple lock and key mechanism.

## VIII. Summary

1. By means of experimentally controlled observations, it has been possible to describe and analyze quantitatively the courtship behavior of the Queen butterfly, *Danaus gilippus berenice*. Laboratory- reared females were released singly to wild Queen males in their natural environment in southern Florida and the courtship behavior was recorded verbally by means of a transistorized tape recorder. A total of 266 courtships of 81 females is analyzed in this paper.

2. The components and phases of successful courtship are summarized in Table 1 and text-figure 9, and the reasons for termination of courtship prior to copulation are in Table 2. The courtship follows the well-known stimulus-response reaction chain. The male pursues the female, overtakes her in the air and induces her to alight on available herbage by rapidly brushing her anterior with two scent-disseminating hairpencils which are extruded from the posterior of his abdomen. If the female is receptive, she acquiesces by folding her wings. The male alights on the female laterally, attempts to copulate and palpates her head alternately with his right and left antenna. Copulation occurs, followed by a post-copulatory flight in which the male carries the female to an inconspicuous area where insemination takes place over a several-hour period during which both remain stationary. If the female is unreceptive during the ground component, the male often successfully reinitiates the entire courtship sequence by fluttering above and alighting on her dorsum.

3. The relationship of the hairpencil glands to the wing glands in male Danainae is considered in detail. On the basis of histological studies, both organs appear to be actively secretory, but only the hairpencils are characteristically odoriferous. The males push their hairpencils into or rub them over their wing glands when they are by themselves, and not engaged in courtships. Seven hypotheses are discussed: (a) that the wing glands produce the scent and the hairpencils disseminate it; (b) the reverse; (c) that both glands produce different active stimulants which play independent roles in the courtship; (d) that the hairpencils produce the scent which both they and the wing glands disseminate at different times in the courtship; (e) that the wing glands produce a secretion which reduces the rate of

evaporation of the hairpencil secretion; (f) that the two secretions combine as a third product which has greater stimulating powers than either alone; and (g) that the wing gland secretion is a preening substance for the hairpencils. Hypotheses (a)-(c) are ruled out, (d) is considered unlikely and limited evidence supports (f). Further work is needed to solve the problem.

4. However, it seems virtually certain that the hairpencils do disseminate the scent about the female. This perfume is regarded as a pheromone which acts as a chemical arrestant of the female's non-specific escape flight from the pursuing male. Presumably it also functions to keep her quiescent after the male has induced her to alight.

5. If the arrestant perfume is species-specific, it may be possible to reconstruct how changes at a molecular level have resulted in the evolution of a sexual isolating mechanism.

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## EXPLANATION OF THE PLATES

### PLATE I

FIG. 1. Dorsal view of the posterior of a male *Danaus gilippus berenice* showing the left and right wing pockets and the tip of the abdomen. The hairpencils are in their retracted position.

FIG. 2. Dorsal view of the posterior of the abdomen and right hindwing of a male *D. g. berenice* with the hairpencils about 75% extruded. **hp** = hairpencil; **shp** = partially evaginated membranous sheath of the hairpencil; **wp** = right wing pocket; **owp** = opening of the wing pocket through which the hairpencil is inserted.

FIG. 3. Hairpencils of *D. g. xanthippus* fully extruded and splayed. The individual hairs arise from the glandular base (**gbhp**) of the hairpencil (see Plate VI, fig. 1) which is here shown completely evaginated; **shp** = fully evaginated membranous sheath of the hairpencil.

FIG. 4. Hairpencils of *D. g. berenice* about 75% extruded.

### PLATE II

FIGS. 1 and 2. A male *D. g. xanthippus* ground hairpencilling (phase 3) the female after having induced her to alight on herbage. In Fig. 1, the hairpencils (**hp**) are fully splayed, whereas in Fig. 2, they are only partially splayed.

FIGS. 3 and 4. Two consecutive slow motion (64 f.p.s.) photographs of a *D. g. berenice* male ground hairpencilling the female (phase 3). In Fig. 3, the hairpencils (**hp**) are partially extruded from the abdomen (**abd**) which has begun to sweep down across the head and antennae of the female. In Fig. 4, 16 microseconds later, the abdomen has completed the sweep; the hairpencils are obscured by foliage.

### PLATE III

FIG. 1. A male *D. g. xanthippus* hairpencilling the female in the air (phase 2). The posterior of the male is between the open wings of the female so that the extruded hairpencils are not visible. Both butterflies are flying rapidly towards the left.

FIG. 2. A male *D. g. xanthippus,* with hairpencils retracted, hovering above the female (phase 4). She clings to herbage, holding her wings outspread in an unreceptive position.

FIG. 3. A male *D. g. xanthippus* attempting to copulate (phase 5) with the female from the right lateral position. Note that the male clings to the undersurface of the female's wings with his legs, while she holds onto the herbage, her wings folded dorsally in the receptive position. The male's abdomen is thrust up between the hindwings of the female.

FIG. 4. A male *D. g. xanthippus* terminating an unsuccessful courtship by desertion.

### PLATE IV

FIG. 1. Copulating pair of *D. g. berenice* with the male clinging to herbage and holding the female upside down at the end of his abdomen.

### PLATE V

FIG. 1. Male *Lycora ceres ceres* held in forceps, showing the large hairpencils spontaneously splayed to the full extent. The left hairpencil is partially hidden from view by the right one.

### PLATE VI

FIG. 1. Median longitudinal section through the glandular base of the hairpencil of a 15-minute post-emergent male *D. g. berenice,* H and E, 100×. **sc** = trichogen secretory cell; **ih** = individual hair of the hairpencil originating in a trichogen cell; **rm** = retractor muscle of the hairpencil; **d** = globular secretion ("dust") between the hairs.

FIG. 2. Globular secretion ("dust") between the hairs of a 3-day post-emergent *D. g. berenice* male, H and E, 400×.

### PLATE VII

FIG. 1. Median transverse section through the *right* wing pocket of a 10-minute post-emergent *D. g. berenice* male, showing how the pocket develops, H and E, 50×. **uwm** = unmodified wing membrane; **Cu2** = second cubitus vein of the hindwing; **fowp** = future opening to the lumen of the wing pocket; **f lumen** = future lumen of the wing pocket; **fcwp** = future cover of the wing pocket; **A** = trichogen secretory cells before expanding to fill nearly the entire space between **A** and **B**.

FIG.  2.  Median transverse section through the mature *left* wing pocket of a 24-hour post-emergent male *D. g. berenice,* H and E, 100×. **uwm** = unmodified wing membrane; (Cu2 is not shown); **owp** = opening to the lumen of the wing pocket through which the hairpencil insertion occurs; **A** = fully expanded trichogen secretory cells nearly filling the space between **A** and **B**; **sc** = individual trichogen secretory cell; **is** = individual specialized flat scales originating in respective trichogen secretory cells. Note that the flat scales are oriented in the direction offering the least resistance to the insertion of the hairpencil.

FIG.  3.  Dorsal view of a wing pocket of a 15-minute post-emergent male *D. g. xanthippus* prior to the folding over of the cover to form the pocket. **Cu2** = second cubitus vein of the hindwing; **fcwp** = future cover of the wing pocket; **fowp** = region of the future opening to the lumen of the wing pocket.

FIG.  4.  Dorsal view of a wing pocket of a 24-hour post-emergent male *D. g. xanthippus* showing the mature organ. **Cu2** = second cubitus vein of the hindwing; **cwp** = cover of the wing pocket; **owp** = region of the opening to the lumen of the wing pocket.

The photographs for Plate I, Figs. 1 and 2, and Plate IV are by Lee Boltin, and the remaining photographs are by the authors and M. A. Jones.